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A quantitative structure-activity relationship (QSAR) study of chlorinated cyclodiene insecticide analogs

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by

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GENERAL INTRODUCTION

Cyclodiene Insecticides

Chlorinated cyclodienes constitute a major class of insecticides which include aldrin, chlordane, dieldrin, endosulfan, endrin and heptachlor. They are produced by the Diels-Alder diene reaction. Chlorination of cyclopentadiene gives hexachlorocyclopentadiene which undergoes a Diels-Alder reaction with an appropriate alkene intermediate to obtain a given compound. The toxicity, chemistry, photochemistry and metabolic fate of the cyclodienes are well defined (1-3). Some undergo oxidative metabolism to form epoxides, and photolysis to yield epoxides and bridged photoisomers, e.g., heptachlor is oxidatively converted to heptachlor epoxide, and each in turn undergoes a photobridging reaction (1) to form photoheptachlor and photoheptachlor epoxide, respectively. Some of the parent cyclodienes and metabolites, e.g., isobenzan and 12-ketoendrin, are highly toxic convulsants. Additionally, conformational isomers exist for some of the chemicals, e.g., aldrin vs. isodrin, dieldrin vs. endrin, β- vs. α-endosulfan, *trans*- vs. *cis*-chlordane. Toxaphene, which is a mixture consisting of 177 different chlorinated bornane derivatives, is a chlorinated alicyclic compound and sometimes is also considered as a cyclodiene-type insecticide (4), though it is not produced by the Diels-Alder reaction, but rather by chlorination of camphene.

Mode of Action for Cyclodiene Insecticides

The action of cyclodienes involves the γ-aminobutyric acid (GABA) binding sites on postsynaptic membranes in the mammalian brain tissue. GABA is a major inhibitory neurotransmitter in the central nervous system (CNS) and at neuromuscular junctions in lower animals (5). This binding involves the GABA receptor ionophore complex (6). Chlorinated cyclodienes bind at the chloride ionophore channel and block the channel, inhibiting the Cl^- flux into the nerve. Ample evidence supporting the hypothesis has come from the biochemical and pharmacological studies using mammalian brain tissue preparations. The GABA receptor is a complex containing at least three binding sites 1) the GABA site, 2) another site where benzodiazepine tranquilizers bind, and 3) a chloride ion ionophore which binds the convulsants picrotoxinin and the bicyclophosphates, as well as the sedative hypnotic barbiturates (7, 8). Activation of GABA receptors increases membrane conductance for Cl^- in postsynaptic membranes, such that when an excitatory impulse is delivered, e.g., by glutamate or acetylcholine, the postsynaptic membrane does not reach threshold, and transmission of the impulse does not occur (5). Picrotoxinin is a polycyclic epoxylactone from seeds of *Anamirta* L. It was a known GABA antagonist as proven by inhibition of GABA-stimulated chloride permeability (9-11). Bicyclophosphorus esters such as *t*-butylbicyclophophorothionate (TBPS) were found in 1970 to 1976 to also be potent convulsants which antagonize the action of GABA (12, 13). α-[8, 10-^{3}H]-Dihydropicrotoxinin ([^{3}H]DHPTX) and [^{35}S]TBPS are highly specific noncompetitive allosteric inhibitors of GABA which bind to the Cl^- ion channel of the GABA complex and

are commonly used as ligands for studying chemicals acting on the receptor (14, 15). Matsumura and Ghiasuddin (16) noted that the cyclodienes inhibit chloride flux and binding of [^{3}H]DHPTX. The cyclodienes also inhibit [^{35}S]-TBPS binding to rat brain membranes (17). Electrophysiological studies with insects and mammals also support the action of the cyclodienes in the GABAergic system (18). Good agreement exists, for the most part, between the potencies of the cyclodienes and hexachlorocyclohexane (BHC) isomers in inhibiting [^{35}S]TBPS binding and their respective mammalian toxicities (19). γ-BHC (lindane), which is not a cyclodiene chemically, is currently considered as a GABA antagonist due to the well-established relationship with the cyclodienes, i.e., cross-resistance and symptomology similarities. However, an interaction of lindane with a putative voltage-dependent chloride channel in electric organ of *Torpedo californica* has also been suggested (20, 21). Mirex and chlordecone, which are chlorinated alicyclic insecticides, but not structurally similar to cyclodienes, act by a different mechanism than the cyclodienes (21).

The GABA receptor in insects is also a molecular target for cyclodienes as indicated by several studies. Picrotoxinin analogs are potent convulsants when applied to desheathed house fly thoracic ganglion (22), though they are nontoxic to house flies when topically applied (23). Cockroaches resistant to γ-BHC and dieldrin were found to be resistant to picrotoxinin (16), and cyclodienes were potent inhibitors of [^{3}H]DHPTX binding to membranes from the nerve cord and brain of the American cockroach (24). The cyclodienes, lindane and toxaphene, showed reduced affinity for the binding sites of GABA receptors prepared from a cyclodiene-resistant house fly strain (25). A cyclodiene-resistant strain of

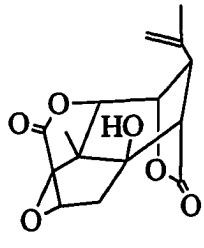
Drosophila melanogaster possessed resistance to dieldrin, aldrin, endrin, picrotoxinin and lindane, but was completely susceptible to DDT, malathion, and propoxur (26). Neurophysiological studies demonstrated that GABA-mediated inhibition in CNS preparations from resistant *Drosophila melanogaster* showed reduced sensitivity to dieldrin or picrotoxinin compared to preparations from susceptible insects (27, 28).

Since the disclosure of the target site for cyclodiene insecticides, new chemicals such as new TBPS and *t*-butylbicycloorthobenzoate (TBOB) analogs, phenylpyrazoles and other bicyclic compounds (29-34) have been synthesized in spite of the existence of extensive insect resistance to these chemicals (35). However, increased understanding of insect and mammalian GABAergic systems may be applied toward improvement of selective toxicity at the target site for better utilization of current and future insecticides.

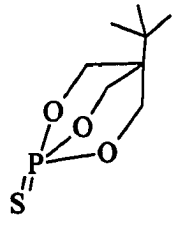
Structure-Activity Relationships of Cyclodiene Insecticides

Structure-activity relationships for the cyclodiene insecticides are still not well understood (36) despite the demise or restricted use for many chemicals from this class of insecticides. As compared with the progress made in the field of biochemical mode of action studies and related pharmacological approaches, research on the structure-activity relationships regarding the interactions between the ligand and the receptor is lagging behind. Furthermore, the readily measurable cellular or membrane responses elicited by ligands are usually secondary consequences of the immediate ligand-induced conformational change, and, clearly, neither the structure, nor even the primary functions of the receptor molecules

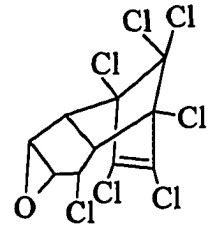
involved, can be deduced from them (37). The classic structure-activity study on chlorinated cyclodienes by Soloway (2), while very extensive, did not address the question of their relationship to the GABA receptor, although he proposed that the bearing of two "electronegative" centers positioned evenly across the plane of symmetry be essential for the insecticidal activity of a cyclodiene compound. Structural similarity to and superimposability of a cyclodiene compound on picrotoxinin or TBPS (see structures below) seemed to be the general basis for explanation of the action at the GABA-chloride ionophore complex (16, 17, 24, 29). Brooks and Mace reported that structural similarity to the carbon skeleton of picrotoxinin and other structural motifs, such as electronegativity of a molecular moiety or atoms, seemed to account reasonably well for insecticidal activity for a series of partially dechlorinated analogs of dieldrin, endrin, endosulfan, isobenzan and photodieldrin (38). However, these explanations of the structure-activity relationships were based on



Picrotoxinin



TBPS



Heptachlor Epoxide

only structural arrangements or superimposability, and the description was qualitative. The quantitation of important structural properties such as electronic, steric, and lipophilic features has not been realized. No quantitative structure-activity relationship (QSAR) study

has been conducted so far since the first discovery of the insecticidal activity of cyclodienes fifty years ago.

Dissertation Objectives

The overall objective of this dissertation research was to conduct a QSAR approach for further understanding and assessing of the dependence of biological action on the physicochemical properties of the cyclodiene insecticides and their analogs, and for predicting the action of structurally related chemicals from this class of insecticides. Specific objectives include: 1) utilize measurements of a biological response descriptor, which can be assigned as a dependent variable of the structural properties of the cyclodiene compounds, and 2) selection of physicochemical parameters as independent variables which could account for the biological action of the compounds, and 3) correlation analysis between the biological information and physicochemical parameters for the cyclodienes.

Methodology

The interactions of pharmacologically active compounds including insecticides with their biological counterparts are determined by intermolecular forces, i.e., by hydrophobic, polar, electrostatic, and steric interactions. QSAR approaches derive models which describe the structural dependence of biological activities either by physicochemical parameters, by indicator variables encoding different structure features, or by three-dimensional molecular property profiles of compounds (39). A QSAR study involves compounds which in their molecular mechanism of action, receptor, enzymes, etc., are analogs. It implies that

compounds bind to largely overlapping domains on their molecular site of action. The study should be restricted to congeneric series of compounds, i.e., three-dimensional homology of various molecular fragments in the chemical framework of the series (40). One of the most widely used techniques for QSAR approaches is the Hansch analysis (39) to derive models in order to describe the structural dependence of biological action on physiochemical properties. Due to the relative structural diversity, i.e., lack of a common parent structure, of the 33 cyclodiene insecticide analogs included in this dissertation research, and the limited availability of using higher level methodology such as the comparative molecular field analysis (CoMFA) (41, 42) when the research project was started, a modified Hansch analysis was used for the investigation involving application of integral parameters (40) for the physicochemical properties. Several physicochemical parameters were employed. The first one was a lipophilicity descriptor which was developed from the retention behavior of the cyclodienes in different mobile phases of a reversed-phase high performance liquid chromatography (RP-HPLC) system. This is a relatively new method which allowed a reliable indirect measurement of the very lipophilic properties of the cyclodiences by using very small amounts of the test compounds (43, 44). Secondly, topological and steric characteristics of the compounds were quantitated by the molecular connectivity method (45-47). The molecular connectivity method is based on topology and graphic theory (45). This method has been broadly used in QSAR analysis (46, 48-51). The third approach focused on the regional electronic effect of the cyclodienes, which was assessed by determining the

chemical shift of the angular proton at the bridge head in proton-NMR spectra, similar to a previous study (52).

The biological descriptor, i.e., the response parameter for the action of the cyclodienes, was measured by an *in vitro* radio-ligand binding bioassay using a rat brain synaptic membrane preparation. The biological data were generated by cooperators at Mississippi State University. A joint grant from the U.S. Air Force provided for them to conduct the biological assays, while I developed the quantitative structure-activity relationships using the chemicals and their biochemical binding assay data. [^{35}S]TBPS was used as the radio-ligand since it appeared to be a better ligand than [^{3}H]DHPTX (14). The assay of this parameter was conducted essentially as described by Casida and Lawrence (29) with slight modification. The potency of each test compound in displacing [^{35}S]TBPS-binding was used as the biological parameter, and was expressed as the concentration inhibiting [^{35}S]TBPS by 50%, i.e., the IC_{50}.

Final data analysis was carried out by multiple regression analyses, using the Statistical Analysis System (SAS 6.07) at a workstation of Project Vincent/ULTRIX v4.3A main frame at the Iowa State University Computation Center (53).

Dissertation Organization

This dissertation incorporates three papers being submitted to scholarly journals, and an appendix presenting chemical synthesis of new biodegradable DDT-type insecticides. Except for the measurement of the biological parameter for chlorinated cyclodiene analogs,

all the work including data collection and analysis and final writing covered in this dissertation was conducted by the author under the advice of Dr. Joel R. Coats. Chapter I, entitled "Determination of lipophilicity of chlorinated alicyclic compounds by reversed-phase high performance liquid chromatography", was published in the *Journal of Liquid Chromatography [Volume 17(9), pp1995-2004].* Chapter II, entitled "Structure-activity relationships of chlorinated alicyclic insecticides", was written in the format for the journal *Pesticide Biochemistry and Physiology* and is being submitted. Chapter III, entitled "A QSAR study on chlorinated cyclodiene insecticides based on their inhibitory activity on TBPS-binding and molecular connectivity", was written in the format for the journal *Pesticide Science* and is being submitted. The General Introduction precedes these three papers, and the Summary and Conclusion follow the papers. The References Cited in the General Introduction follow the General Summary and Conclusion. An appendix section, entitled "Directed synthesis of new biodegradable DDT-type insecticides" was included at the end as an independent section of the dissertation, but it was still related to the main topic to some extent.

CHAPTER I. DETERMINATION OF LIPOPHILICITY OF CHLORINATED ALICYCLIC COMPOUNDS BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

A paper published in *Journal of Liquid Chromatography*

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ABSTRACT

A RP-HPLC procedure has been developed for measuring the capacity factor (k') of a series of chlorinated alicyclic compounds. The chromatographic behavior measured on a 4.5 mm i.d. x 3.3 cm C-18 column with methanol/water as the mobile phase was related to the volume fraction of methanol (ϕ). A linear relationship was found between log k' and ϕ, showing correlation coefficients of $r > 0.99$, for each of the 15 chlorinated alicyclic compounds tested. The log k_w, the capacity factor obtained by extrapolation of the retention data from binary eluents to 100% water, was chosen as a measure of the solute lipophilicity. Since log k_w is considered as a valuable index of the lipophilicity of a compound, the determined values will be

used for quantitative structure-activity relationship studies of the chlorinated alicyclic compounds.

INTRODUCTION

The lipophilicity of a bioactive molecule is one of the most important physicochemical properties which influences its capacity to move through biological compartments. It is generally defined as the tendency of a chemical to distribute between an immiscible nonpolar solvent and water. The logarithm of the partition coefficient of a chemical in the *n*-octanol/water system (log K_{ow}), which is usually measured by 'shake-flask' method, is widely used because of its simplicity and some similarity between *n*-octanol and biological membranes. The 'shake-flask' method works in most cases, but it results in large errors for a chemical with a log K_{ow} larger than 4, and the procedure is time-consuming and requires considerable amounts of pure stable compounds [1]. It has been proven that the retention capacity factor (*k'*) of a compound in a reversed-phase high performance liquid chromatography (RP-HPLC) system is a reliable indirect descriptor of the lipophilicity of a compound [1-5, 10-16]. Moreover, recent studies have shown that log k_w, the retention capacity factor which was extrapolated from a binary phase to 100% water in a RP-HPLC system, is an even better descriptor of lipophilicity than the isocratic factor [4-5,10-11].

The chlorinated alicyclic compounds, which were insecticides widely used in the past including aldrin, dieldrin, heptachlor and their structural analogs, constitute a large group of

compounds which are environmentally and toxicologically important [6-7]. Their neurochemical action occurs through binding to the chloride channel at the γ-aminobutyric acid (GABA) receptor [6]. These compounds are generally very non-polar; for example, aldrin has a log K_{ow} as high as 5.9 [9]. Unfortunately, few log K_{ow} values have been documented for this class of compounds. Direct measurement of their *n*-octanol/water partition coefficients by the conventional 'shake-flask' method is difficult because of their highly lipophilic characteristics and the availability of adequate amounts for the measurement. In this paper, we are reporting a systematic study of the lipophilicity of these compounds by using a RP-HPLC method, and the measured data will be used in an on-going research of quantitative structure-activity relationships (QSAR) for the compounds.

MATERIALS AND METHODS

Chemicals. The purity for each of the 15 chlorinated alicyclic compounds was greater than 98%. The structures of the compounds were further confirmed by proton-NMR spectra. A stock solution of each compound was made at a concentration of 1 mg/ml in methanol and stored at -20 °C. All other chemicals and solvents were analytical reagent or HPLC grade.

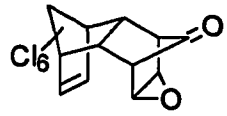
Apparatus and Chromatographic Conditions. The RP-HPLC system consisted of a Waters 6000A pump coupled with a U-6K injector, a 4.5 mm i.d. x 3.3 cm C-18 analytical column with a particle size of 3 microns, which was manufactured by Perkin-Elmer Corp., Norwalk, Connecticut, a variable-wave-length ultraviolet detector (Spectroflow 757, ABI

Analytical Kratos Division, Ramsey, New Jersey), which was set at 210 nm or 220 nm, and a recorder (Cole-Parmer Instrument Company, Chicago, Illinois).

Measurement of log *k'*. The dead volume of the system was measured by injecting a 10% $NaNO_2$ solution. The stock solutions of the tested compounds were diluted with methanol to the final injection concentration around 100 μg/ml. A 15-μl injection was made in triplicate. According to their chromatographic behavior, the retention times were determined at five different methanol/water eluent ranged from 60% to 80% of methanol by volume. At each mobile phase composition, the capacity factor was calculated according to $k' = (t_R - t_0)/t_0$, where t_R and t_0 were the retention times of the analyte and of the non-retained compounds respectively. The log k_w values, were obtained from the y-intercept of the plots of log *k' versus* volume fraction of methanol in the mobile phase.

RESULTS AND DISCUSSION

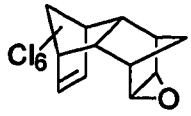
The structural information of the 15 tested compounds is given in Figure 1. Chromatographs of all tested compounds were accomplished under a variety of conditions in which the volume fraction of methanol (ϕ) in the mobile phase varied from 0.60 to 0.80, since smaller fractions of this component led to unreliable and long retention times. Most of the chlorinated alicyclic compounds are very hydrophobic. This attribute results in unreliably long retention times and trailing of separations by using the commonly used C-18 columns (10 cm or 25 cm in length). However these obstacles were eliminated by introducing a 4.5 mm i.d. x 3.3



1. 12-Ketoendrin

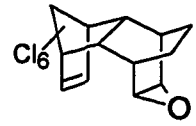
2. Chlordene epoxide

3. Heptachlor epoxide

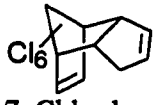


4. Endrin

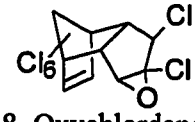
5. Dieldrin



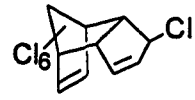
6. Epoxide of 14



7. Chlordene



8. Oxychlordane



9. Heptachlor

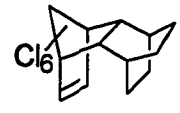
10. Dihydrochlordene

11. Isodrin

12. @

13. Aldrin

14. @@



15. Octahydro-derivative of 14

Figure 1. Structures of the chlorinated alicyclic compounds.

@: Hexachlorocyclopentadiene/cyclohexa-1,3-diene adduct
@@: Hexachloronorbornadiene/cyclohexa-1,3-diene adduct

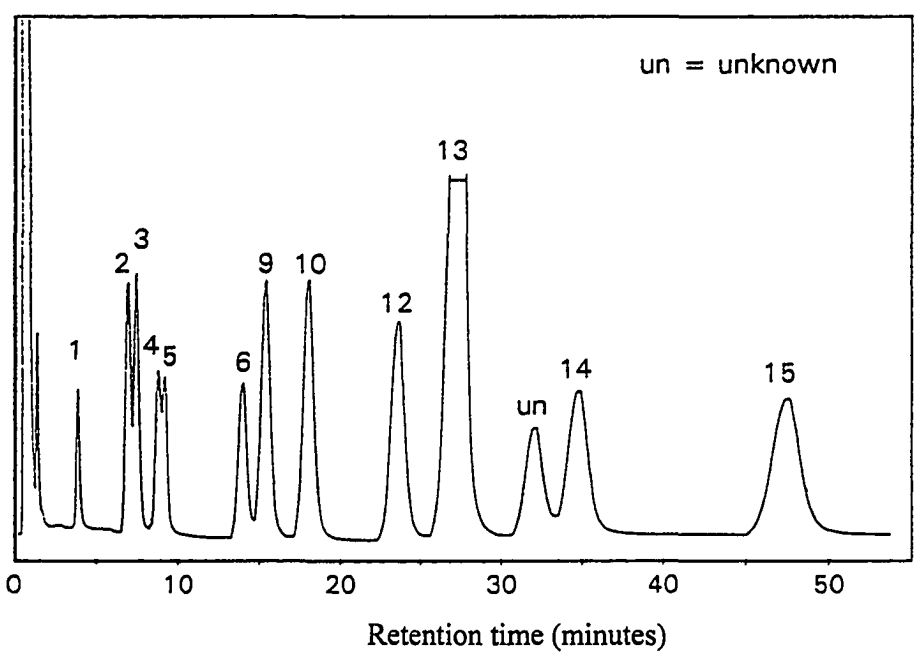


Figure 2. HPLC profile of the chlorinated alicyclic compounds. Column: 4.5 mm i.d. x 3.3 cm C-18 cartridge pack; Mobile phase: 70/30 methanol/water + 0.01% H_3PO_4; Flow rate: 1.0 ml/min; Detector: UV-210 nm; Temp.: 25 °C.

cm C-18 analytical column packed with 3-micron support, and this allowed the tested compounds to be eluted at a reasonable time even in the case of the most polar mobile phase. Separations were improved by adding a trace amount of phosphoric acid at a concentration of 0.01% by volume to the mobile phases, and the reproducibility of retention behavior was not affected for the tested compounds (see Figure 2).

Retention capacity factors (k') at each methanol fraction are given in Table 1 for the 15 tested compounds. Although the monocratic log k' values are possibly correlated to other lipophilic descriptors, the established log k' - lipophilicity correlation for a given class of compounds cannot be extrapolated either to different solutes or to other similar or even identical separation systems, and it may result in misleading data owing to solute-solvent interactions [4].

The log k_w, the retention capacity factor of a compound when 100% water was employed as mobile phase in a RP-HPLC system, was used for evaluating the lipophilicity of the compounds because it eliminated selective solute-solvent interactions and was more closely related to log K_{ow} than isocratic capacity factors [4, 12-16]. The log k_w was determined by extrapolating the polycratic retention capacity factors (log k's) from binary eluents to 100% water. It was found that for the 15 tested compounds, the relationship between solute retention capacity factor and the composition of methanol in the mobile phase can be described by the equation:

$$\log k' = \log k_w - S\,\phi \quad (1)$$

Table 1. Isocratic retention capacity factors (k') of the chlorinated alicyclic compounds.

Compound	ϕ: Methanol/water (v/v)				
Number	0.60	0.65	0.70	0.75	0.80
1	22.737	11.562	7.342	3.991	2.389
2	46.286	23.366	14.081	6.887	4.253
3	53.571	26.158	15.211	7.695	4.141
4	63.395	30.921	18.363	9.296	5.286
5	71.150	33.406	19.443	9.620	5.252
6	112.514	52.522	30.467	14.946	8.020
7	120.974	54.229	28.844	14.345	7.594
8	136.601	59.478	30.662	14.495	7.216
9	136.601	60.777	33.650	14.956	7.722
10	161.867	71.660	39.641	18.238	10.103
11	220.067	87.790	50.001	20.690	11.278
12	222.032	96.616	52.047	21.391	12.224
13	267.860	113.063	60.476	24.074	13.832
14	357.519	149.764	78.426	29.466	17.502
15	517.449	210.398	108.462	43.807	20.961

Table 2. Linear relationship between log k' and ϕ:
log $k' = \log k_w - S\,\phi$

Compound	S	log k_w	r^2 (n = 5)*
1	4.84	4.24	0.9970
2	5.21	4.78	0.9965
3	5.51	5.02	0.9987
4	5.36	5.00	0.9979
5	5.61	5.20	0.9980
6	5.68	5.44	0.9980
7	5.96	5.64	0.9983
8	6.34	5.92	0.9989
9	6.21	5.85	0.9980
10	6.01	5.79	0.9971
11	6.42	6.16	0.9945
12	6.35	6.14	0.9956
13	6.49	6.30	0.9948
14	6.70	6.55	0.9939
15	6.93	6.86	0.9989

* r = Correlation coefficient.

where S refers to the slope of log k' *vs.* ϕ plots. The correlation coefficients ($r > 0.99$) showed that log k' and ϕ were highly linearly correlated for the 15 compounds. The slopes for the equations were in the range of 4.84 to 6.93. The extrapolated log k_w values are given in Table 2. The relationship between the slope S and the intercept values (log k_w) was investigated for the tested compounds. A good linear correlation was observed: correlation coefficient = 0.9937. Slope S depends on the size of the solute molecule and the structure of polar functional groups. The high linear correlation coefficient may be a reflection of the uniqueness and suitability of the methanol-water system for estimating the lipophilicity of the compounds [4].

CONCLUSIONS

The retention capacity factor (log k') of a compound in a RP-HPLC system can be used as a descriptor of its lipophilicity. The isocratic log k' was measured at five different compositions of the eluent, and the log k_w was extrapolated from the linear relationship between log k' and the fraction of methanol in the mobile phase for each of the 15 chlorinated alicyclic compounds. The log k_w values may be advantageous over other parameters in describing the lipophilic properties of the structurally related, very nonpolar, chlorinated alicyclic compounds, in QSAR studies.

AKNOWLEDGEMENTS

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CHAPTER II. STRUCTURE-ACTIVITY RELATIONSHIPS OF CHLORINATED ALICYCLIC INSECTICIDES

A paper being submitted to *Pesticide Biochemistry and Physiology*

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ABSTRACT

Quantitative structure-activity relationships (QSAR) between the inhibitory effect of specific *t*-butylbicyclophosphorothionate (TBPS) binding to rat brain P2 membrane, and the lipophilic parameter log k_w, which was developed by a reversed-phase high-performance liquid chromotagraphic system, and the first-order valence molecular connectivity index, $^1\chi^v$, were studied for 27 chlorinated alicyclic insecticides such as heptachlor, aldrin and their structural analogs. This study showed that lipophilicity is very important to their biochemical toxicity and the epoxide or ketone structural congeners and the non-epoxide non-ketone analogs may bind to different regions, respectively, on the common GABA receptor. The epoxide or ketone congeners may bind at a hydrophilic region, and a

negatively correlated linear relationship exists between the inhibition of TBPS-binding and lipophilicity. However, the non-epoxide non-ketone analogs may bind at a lipophilic region, and there is a positively correlated linear relationship between them. It seems that the epoxide group is an essential structure feature of the chlorinated cyclodienes for eliciting their high inhibitory activity at the GABA receptor. The first-order valence molecular connectivity index is linearly correlated very well with the activity on the inhibition of TBPS-binding for the compounds tested, when the 22 chlorinated alicyclic compounds of closest structural similarity were used.

INTRODUCTION

Chlorinated alicyclic compounds including lindane, aldrin, heptachlor and their structural analogs, representing a major group of insecticides used in the past, have been purported to act as noncompetitive antagonists of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) receptor [1-5]. They bind at the picrotoxinin site, which is also specific to the cage convulsant *t*-butylbicyclophosphorothionate (TBPS) at the GABA-chloride ionophore complex [6, 7]. Many chlorinated alicyclic insecticides have been shown to bind at this site [2, 4, 8]. Thus the effect of chlorinated alicyclic insecticides is mediated by competing for the binding site on the GABA receptor-chloride ionophore complex, suppressing activity of the GABA-ergic inhibitory neurons and resulting in hyperexcitation.

However, structure-activity knowledge for the chlorinated alicyclic insecticides is not well developed as compared with the progress made in the field of biochemical mode of action studies. The classic structure-activity study on chlorinated cyclodienes by Soloway [9], while very extensive, did not address the question of their relationship to the GABA receptor. The degree of similarity between a compound and picrotoxinin seems to correlate with its potency at the GABA-chloride ionophore complex, but the similarity is based on only the superimposability of these structures [5]. Brooks and Mace reported that structural similarity to the carbon skeleton of picrotoxinin and its center of electronegativity seemed to account reasonably well for insecticidal activity for a series of partially dechlorinated analogs of dieldrin, endrin, endosulfan, isobenzan and photodieldrin [10]. The similarity to the specific binding ligand TBPS can also be an indication of potency in preparations from mammalian brains [5]. However, these structure-activity studies were only qualitative; the quantitation of the most important structural parameters, those which code physicochemical properties of the compounds, has not been realized.

In this paper, a study of quantitative structure-activity relationships (QSAR) is presented for up to 27 chlorinated alicyclic compounds (insecticides and their structural analogs) by using simple linear regression analysis between their biological activities and two physicochemical parameters. The median inhibitory concentration (IC_{50}) of the chlorinated alicyclic insecticides for competing with the binding of TBPS is used as the biological parameter in this study. The two physicochemical parameters employed are (1) the lipophilic parameter, $\log k_w$, which was developed through a reversed-phase high-performance liquid

chromotagraphic system, and (2) the first-order valence molecular connectivity index, $^{1}\chi^{v}$, which is an indicator of topology and branching of the carbon skeleton. The investigation focuses on the relationships between the individual parameters and the competitive binding activity at the site of action, to explain the variation in the biological activity among the insecticidal analogs. The study may help describe in more detail the binding site, as well as provide a predictive capability for toxicological effects of other analogs in this category.

MATERIALS AND METHODS

Chemicals.

Most of the compounds listed in Table 1 were obtained from Velsicol Chemical Corporation and Chem Service, Inc. Some of the structural analogs were synthesized by Dr. Earl Alley at Mississippi State University. The purity of each compound was > 98% as determined by thin-layer chromatography. Ligands, [^{35}S]TBPS and unlabelled TBPS, were purchased from New England Nuclear Corporation. Their purity was checked by thin-layer chromatography and was > 99%.

Biological parameter.

The potency of each test compound in displacing [^{35}S]TBPS-binding in a rat brain synaptic membrane preparation was used as the biological parameter, and was expressed as

the concentration that inhibits [^{35}S]TBPS by 50%, i.e., the IC_{50}. The assay of this parameter was conducted essentially as described by Casida and Lawrence [5] with slight modification.

Membrane preparation. Following decapitation, the female rat (*Rattus norvegicus*, Sprague-Dawley strain [Cr l: CD(SD)BR]) brains were homogenized in 50 volumes of 1 mM ethylenediamine tetraacetic acid (EDTA). The P2 fraction was isolated by centrifuging at 1,000 x *g* for 10 minutes. The supernatant was centrifuged at 9,000 x *g* for 20 minutes. The pellet was resuspended in 1 mM EDTA and dialyzed 3 times, for 2 hours each time, against 20 volumes of distilled, deionized water to remove endogenous ligands. The suspension was sedimented at 25,000 x *g* for 30 minutes. The resulting pellet was finally suspended in assay buffer. Protein was determined by the Coomassie Brilliant Blue G method [11].

Inhibition of [^{35}S]TBPS-binding. The membrane suspension which was equivalent to 0.25 mg protein was incubated with 2.0 nM [^{35}S]TBPS and a test compound in 5 µl dimethyl sulfoxide (DMSO) in assay buffer (1.0 ml) at 37 °C with shaking for 30 minutes. The incubation was terminated by adding 5 ml of cold buffer, followed by rapid filtration onto glass microfilter paper. Two additional rinses with 5 ml of chilled buffer followed. The filter paper was shaken for 1 hour with a liquid scintillation cocktail in a scintillation vial and kept in the dark overnight. Radioactivity was measured with a Beckman LC-3801 liquid scintillation counter. A series of concentrations of each test compound was employed to determine its potency, i.e., the IC_{50}. Each concentration was run in triplicate. The control tubes received 5 µl DMSO only.

Physicochemical parameters.

Lipophilic parameter, Log k_w. The log k_w value of a compounds was used as an index of lipophilicity in the study of the structure-activity relationships. The log k_w value was extrapolated from the capacity factor log k which was evaluated from retention time in a reversed-phase HPLC system. Theoretically, this lipophilic parameter represents the partitioning tendency between the hydrophobic stationary-phase and 100% water in a reversed-phase HPLC system and is advantageous over other lipophilic descriptors [12, 13]. Detailed methods for measurement of this parameter was reported in a previous paper [13]. The log k_w values are listed in Table 1, along with the biological parameters of the compounds.

Molecular connectivity index. The first-order valence molecular connectivity index ($^1\chi^v$) was another physicochemical parameter used for investigating the structure-activity relationships. $^1\chi^v$ is the sum of the total one-bond terms in a compound, and it is calculated according to following formulas:

$$^1\chi^v = \sum(\delta_i^v \bullet \delta_j^v)^{-0.5}$$

$$\delta^v = \frac{Z^v - h}{Z - Z^v - 1}$$

where Z is the atomic number, Z^v is the number of valence electrons in an individual atom and h is the number of hydrogen atoms bonded to that atom. Comprehensive discussions of

molecular connectivity methodology have been published by Kier and Hall [14, 15]. The first-order $^1\chi^v$ contains topological information in terms of one-bond fragments which form a given compound. A given compound has its unique $^1\chi^v$ value. Calculated $^1\chi^v$ values for the compounds are given in Table 1.

RESULTS AND DISCUSSION

Activity on inhibition of TBPS binding

Activities on inhibition of TBPS-binding for 27 chlorinated alicyclic compounds, which are expressed in log ($1/IC_{50}$), are given in Table 1. The unit of IC_{50} is nanomolar concentration. The results show that the log ($1/IC_{50}$) values range from -0.635 to -4.267. The most potent among them are 12-ketoendrin, photoheptachlor epoxide and Δ-ketoendrin, while aldrin *cis*- and *trans*-diols and chlordecone have extremely low activity. There is no detectable activity observed for mirex. Lindane has a moderate activity in the assay. Dieldrin and endrin, a pair of stereoisomers, show a thirteen-fold difference in their activities while aldrin and isodrin, another pair of stereoisomers, show an eleven-fold difference in activity. Additionaly, the epoxide is more potent than its parent cyclodiene form, for example, three pairs of compounds that demonstrate this pattern are dieldrin and aldrin, heptachlor epoxide and heptachlor, chlordene epoxide and chlordene.

Lipophilicity vs. IC_{50} for TBPS-Binding

Lipophilicity plays an important role in QSAR in two different ways. One is the 'substituent contribution', the regional contribution to lipophilicity involved in the interaction of a compound with its specific binding domains, and the other is the 'integral lipophilicity' represented by the aggregate hydrophobic fragmental constant considering a molecule as a whole [16]. The latter, the overall lipophilicity descriptor, is of particular significance for mechanisms based on passive transport, such as those involved in the blood-brain barrier passage, tissue uptake, passive renal reabsorption, etc., and it also contributes to the binding of drug molecules to receptors, enzymes, plasma proteins, etc. [17]. This is not only related to the lipophilicity in particular regions of the molecule critical in its interaction with particular binding domains, but also to the shift of the active molecule as a whole from the free water phase to the surface of biological macromolecules and structures such as membranes.

Log k_w, the lipophilic descriptor used in this study, which is parallel to the logarithm of the partition coefficient between 1-octanol and water [12, 13], is clearly a parameter expressing the overall lipophilic characteristics of a molecule. The values of Log k_w and the activity on inhibition of TBPS-binding are shown in Table 1. Because of the relative diversity of the compounds, log k_w and log ($1/IC_{50}$) do not show a significant relationship statistically when they were first treated as a single group. However, two patterns or relationships were observed after the compounds were sorted into two groups, Group I and Group II, according to their structural congenerity.

Compounds in Group I (Figure 1-A) contain epoxide or ketone functional groups. The relationship shows that the activity as inhibitors of TBPS-binding of the compounds increases as they become more hydrophilic. This negative linear relationship is described as follows:

$$\text{Group I: } \text{Log}\,(1/IC_{50}) = -1.29\,\text{Log}\,k_w + 4.71 \quad n = 9, r = 0.729 \quad (1)$$

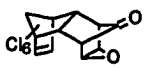
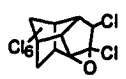
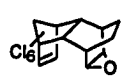
Structurally, all of the compounds in Group I bear oxygen atoms, and all of these compounds have an epoxide group except Δ-ketoendrin, which is a ketone. The epoxides are markedly more potent than their parent form, for example, dieldrin and aldrin, and heptachlor epoxide and heptachlor.

In contrast to Group I, the compounds in Group II, showed that log ($1/IC_{50}$) values increase as they become more hydrophobic, and a positive linear relationship existed among them (Figure 1-B). None of these compounds in Group II bears an epoxide or ketone group. Compounds such as aldrin *cis*- and *trans*-diols are among the compounds with the lowest potency, perhaps due to their poor lipophilicity.

$$\text{Group II: } \text{Log}\,(1/IC_{50}) = 0.983\,\text{Log}\,k_w - 8.42 \quad n = 14, r = 0.872 \quad (2)$$

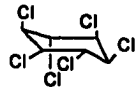
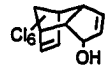
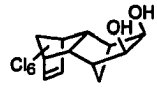
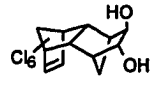
The opposite relationships in Group I and II compounds between lipophilicity and activity on inhibition of TBPS-binding may indicate specific ligand-receptor interactions, since the activity measurement was an *in vitro* assay in this study, and the complicating influences of penetration, metabolism, and excretion were eliminated. These opposite relationships may indicate that Group I and Group II compounds might bind in different ways or to different regions respectively on the common binding site. The data indicate that the epoxide form of chlorinated cyclodienes may be an essential structure feature for eliciting the highest inhibitory

Table 1. Relationship between the first-order valence molecular connectivity index ($^1\chi^v$), and a lipophilic parameter (log k_w) and inhibition of specific [^{35}S]-TBPS binding to rat brain P2 membrane by chlorinated alicyclic compounds.

No.	Name	Structure	Log k_w Ref.[11]	$^1\chi^v$	Log ($1/IC_{50}$)	Log ($1/IC_{50}$) Calculated	Residual
1	12-Ketoendrin		4.240	8.482	-0.635	-0.485†	0.150
2	Photoheptachlor epoxide		4.452$^{@}$	8.286	-0.723	-0.926†	0.203
3	Δ-Ketoendrin		4.525$^{@}$	8.614	-1.068	-1.495‡	0.427
4	A*		6.860	8.862	-1.118	-1.052‡	0.066
5	Endrin		5.002	8.517	-1.231	-1.668‡	0.437
6	Isodrin		6.162	8.046	-1.467	-1.466†	0.001
7	Photo-oxychlordane		5.037$^{@}$	8.768	-1.401	-1.220‡	0.181
8	Dihydroisodrin		6.447$^{@}$	8.362	-1.700	-1.945‡	0.245
9	B*		6.554	8.546	-1.767	-1.617‡	0.150
10	Heptachlor epoxide		5.023	8.022	-1.822	-1.520†	0.302
11	Dihydroaldrin		6.529$^{@}$	8.362	-2.181	-1.945‡	0.236
12	C*		5.443	9.017	-2.093	-0.776‡	1.317

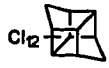
(Table 1 Continued)

(Table 1 Continued)

13	Dieldrin		5.200	8.517	-2.346	-1.668‡	0.678
14	Lindane		3.795	5.928	-2.268	-	-
15	Photochlordene		4.800@	7.623	-2.301	-2.417†	0.116
16	Heptachlor		5.848	7.550	-2.545	-2.580†	0.035
17	Aldrin		6.301	8.046	-2.535	-2.510‡	0.025
18	2,3-Chlordene epoxide		4.776	7.517	-2.480	-2.656†	0.176
19	D*		6.136	7.546	-2.629	-2.591†	0.038
20	E*		6.506@	7.879	-2.769	-2.807‡	0.038
21	Dihydro-chlordene		5.791	7.379	-3.245	-2.966†	0.279
22	Chlordene		5.638	7.046	-3.405	-3.716†	0.311
23	1-Hydroxyl chlordene		4.328@	7.154	-3.726	-3.472†	0.254
24	Aldrin *cis*-diol		4.574@	8.562	-3.967	-	-
25	Aldrin *trans*-diol		4.418@	8.562	-4.125	-	-
26	Chlordecone		4.541@	9.374	-4.267	-	-

(Table 1 Continued)

(Table 1 Continued)

27	Mirex		7.388[@]	-	No inhibition	-	-

* A, 5,6,7,8,9,9,-hexachloro-1,2,3,4,4a,5,8,8a-octahydro-1,4-ethano-5,8-methanonaphthalene;

B, 5,6,7,8,9,9,-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-ethano-5,8-methanonaphthalene;

C, 5,6,7,8,9,9,-hexachloro-1,2,3,4,4a,5,8,8a-octahydro-2,3-epoxy-1,4-ethano-5,8-methanonaphthalene;

D, 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-1,4-methanonaphthalene; E, 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,8a-hexahydro-1,4-methanonaphthalene.

[@] Unpublished data.

† Predicted from Equation-4.

‡ Predicted from Equation-5.

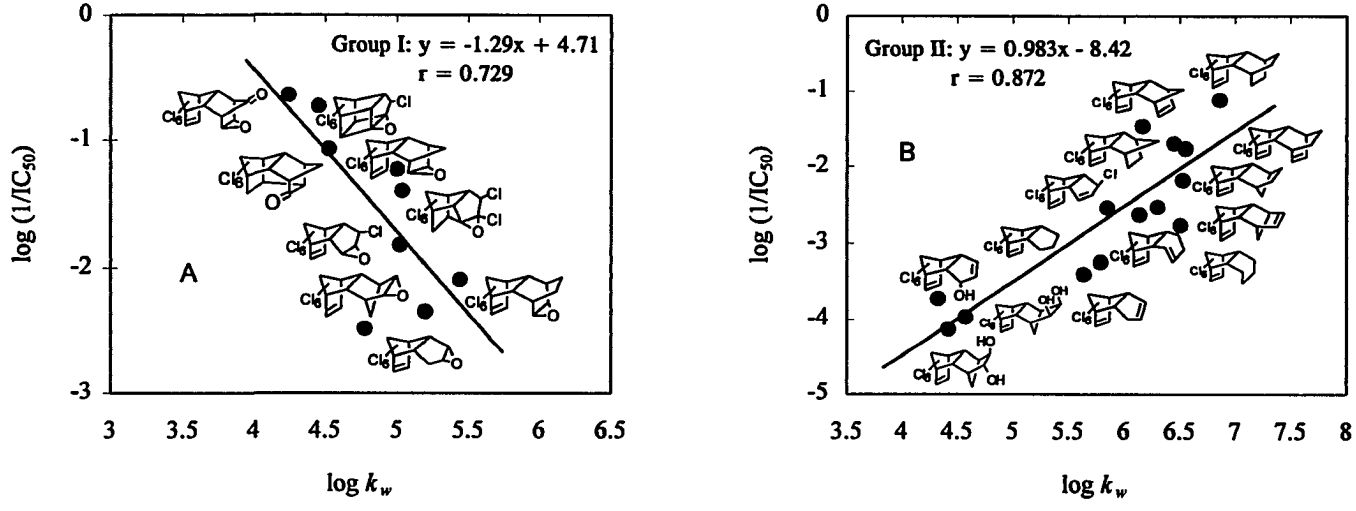


Figure 1. The relationship between the lipophilic parameter, log k_w, and IC_{50} for chlorinated cyclodiene compounds: Group I = epoxide and ketone congeners, and Group II = non-epoxide congeners.

activity at the GABA receptor. The alkene form of the parent compound can be converted to the epoxide form by bioactivation metabolism *in vivo*. It has been reported that the coincidence of biotransformation of heptachlor, aldrin, and isodrin to their epoxides in the tissues of the treated insects with the onset of the poisoning symptoms was observed [18]. Compounds in Group II do not bear the epoxide group, and their action may partially be effected by interaction with membranes or a lipophilic region of the GABA receptor. For the compounds that are more lipophilic, a stronger affinity may occur at the binding region, and result in more damage to the integrity or flexibility of the receptor.

Before the linear regression analysis was conducted, lindane, chlordecone and photochlordene were trimmed as outliers based on the structural comparison to other compounds (cyclodienes) in the data set. Obviously, lindane lacks structural congenerity as compared to other compounds, the products of the Diels-Alder diene reactions and their photo-rearrangement analogs. Although current hypotheses seem to indicate that lindane acts primarily at the GABA receptor site [4,19, 20], Eldefrawi *et al.* reported that lindane also displaces competitively the [^{35}S]-TBPS to bind to a putative voltage-dependent chloride channel of *Torpedo*, and the affinity of lindane for these putative voltage-dependent channels is higher than that for the $GABA_A$ receptor [21, 22]. Both chlordecone and photochlordene are cage-like compounds, and they also lack congenerity as compared to other compounds in Group II, and they were trimmed as outliers when sorting the data. Mirex has no inhibitory effect on TBPS binding, and chlordecone has very low potency in this study. Although chlordecone inhibits binding of [^{35}S]-TBPS to the putative voltage-dependent chloride channel,

mirex does not [22]. Since these two insecticides share some of their toxicity symptoms with lindane, and mirex may be metabolically converted to chlordecone, it has been speculated that voltage-dependent chloride channels may also play a role in the action of these insecticides [22]. This current project has shown chlordecone and mirex to be essentially inactive and lindane to be only moderately active.

The First-Order Valence Molecular Connectivity vs IC_{50} of TBPS Binding

Molecular connectivity indices have been shown to be rich in structural information related to topological, geometric and spatial attributes of a compound [14, 15]. They are often considered as descriptors of the size, degree of branching, unsaturation, cyclicity and heteroatom content for the molecules in a series. For the compounds included in this study, the first-order valence molecular connectivity index has shown significant linear relationships with the activity on inhibition of TBPS-binding for most of the 27 compounds studied, indicating the predictive power of the molecular connectivity index (Equation 3 and Figure 2).

$$\text{Log}\,(1/IC_{50}) = 1.15\ {}^{1}\chi^{v} - 11.4 \quad n = 22,\ r = 0.771, \quad (3)$$

The first-order valence molecular connectivity index, ${}^{1}\chi^{v}$, gives weight to structural features of one-bond-length fragments in the molecules. The positive linear relationship indicates that with increasing number of atoms, the inhibitory effect of a compound also increases, and also with an increase in the heteroatom content of a compound, the inhibitory effect increases.

Aldrin *cis*- and *trans*-diols (No. 24 and 25) were trimmed from the original set of 27. The reason for eliminating these two chemicals is that they have extremely low activity in the *in*

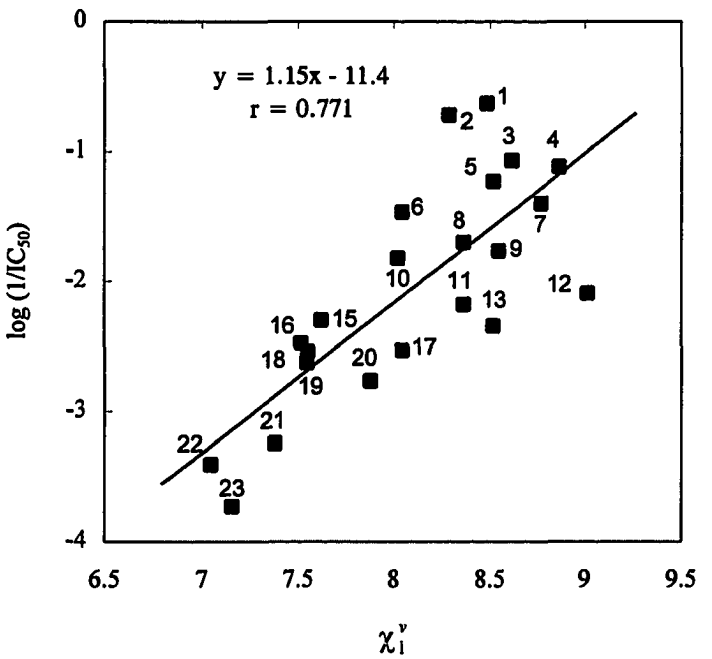


Figure 2. The relationship between the first-order valence molecular connectivity index and IC_{50} for 22 chlorinated cyclodiene compounds.

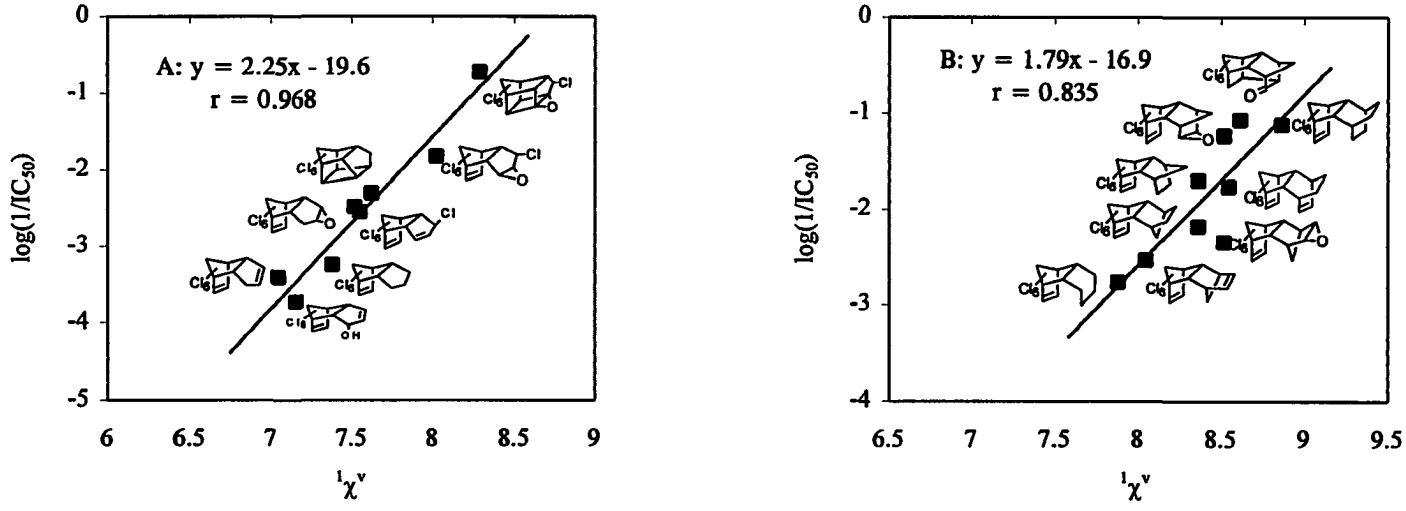


Figure 3. The relationship between the first order valence molecular connectivity index and IC_{50} for chlorinated cyclodiene compounds: A = heptachlor-related congeners, and B = aldrin-related congeners.

vitro assay, as do chlordecone and mirex. These four compounds can essentially be considered inactive in binding competitively with TBPS. Again, lindane, a cyclohexane, is dropped from the series of compounds because all other members are cyclodiene congeners. The first-order valence molecular connectivity index gives a more precise prediction on the inhibitory activity of TBPS binding for the compounds when the compounds are further divided into two groups, heptachlor-related and aldrin-related congeners (Figure 3).

Heptachlor congeners: $\text{Log}\,(1/IC_{50}) = 2.25\ {}^{1}\chi^{v} - 19.6 \quad n = 8, r = 0.968 \quad (4)$

Aldrin congeners: $\text{Log}\,(1/IC_{50}) = 1.79\ {}^{1}\chi^{v} - 16.9 \quad n = 9, r = 0.835 \quad (5)$

By using Equations 4 and 5, the calculated IC_{50} values for the compounds are given in Table 1. The residual of these predictions has a range from 0.001 to 0.678 with only one exception, which is compound No. 12 with an extreme deviation value of 1.317 between the experimental and predicted values. In summary, the first-order valence molecular connectivity index correlated well with binding at the site of action, if the series is limited to the 22 chlorinated cyclodiene compounds of close structural similarity.

In conclusion, lipophilicity plays an important role for the action of the chemicals studied. Log k_w might be a useful probe for elucidating the ligand/receptor interactions at the site of action. The first-order valence molecular connectivity index, ${}^{1}\chi^{v}$, is a powerful predictive index for structurally related compounds, and it can be used to predict the toxic effect of similar compounds.

ACKNOWLEDGMENT

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CHAPTER III. A QSAR STUDY ON CHLORINATED CYCLODIENE INSECTICIDES BASED ON THEIR INHIBITORY ACTIVITY ON TBPS-BINDING AND MOLECULAR CONNECTIVITY

A paper being submitted to *Pesticide Science*

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ABSTRACT

A quantitative structure-activity relationship (QSAR) study is presented on the inhibitory activity of 22 chlorinated cyclodiene insecticide analogs on specific *t*-butylbicyclophosphorothionate (TBPS) binding to rat brain P2 membrane by using the orthogonal molecular connectivity index descriptors. The orthogonal descriptors were derived from the first-, second-, third- and fourth valence molecular connectivity indices, *i.e.*, $^{1}\chi^{v}$, $^{2}\chi^{v}$, $^{3}\chi^{v}$, and $^{4}\chi^{v}$. The descriptors showed a satisfactory predictability for the biological parameter: Log $1/IC_{50} = 1.35(\pm 0.292)^{1}\Omega + 0.487(\pm 0.316)^{2}\Omega + 1.32(\pm 0.651)^{4}\Omega - 12.9(\pm 2.37)$ [$n = 22$, $r = 0.934$, $s = 0.344$, $F_{3,18} = 40.1$]. Regression analysis showed that employing

the orthogonal descriptors adds stability in the model, and interpretation of the individual effect from each prediction descriptor is unique and without ambiguity.

INTRODUCTION

Widespread interest in chloride channel ion antagonists as offering directions for new insecticides has refocused attention on the mechanism of toxic action of the chlorinated cyclodienes and their structural analogs [1-5]. The action of chlorinated cyclodiene insecticide analogs is due to their ability to block the γ-aminobutyric acid (GABA)-gated chloride ion channel on the GABA receptor ionophore complex [1, 2]. Evidence supporting this mechanism of action comes from radiolabelled-ligand studies showing the inhibitory effect of chlorinated cyclodienes on the binding of [^{3}H]dihydropicrotoxinin and [^{35}S]*tert*-butylbicyclophosphorothionate (TBPS) to the GABA receptors [2, 3]. However, the structure-activity relationships for this class of compounds are not well developed. The interpretation of activity of a compond previously has been solely based on the degree of its structural similarity to picrotoxinin and TBPS analogs, and this similarity is judged only on the superimposability of these structures [2, 4, 5]. We have recently reported on basic regression analyses with chlorinated cyclodienes [6]. So far there is no multiple-regression quantitative structure-activity relationship (QSAR) study reported for chlorinated cyclodiene analogs.

Since the algorithm of molecular connectivity was first introduced by Randić [7], it has been extensively developed by Kier and Hall and used in QSAR approaches with many classes of biologically active molecules [8-10]. A pool of topological indices can be calculated for molecular connectivity, which encode information about size, branching, cyclization, unsaturation and heteroatom content of a molecule. But due to the closely interrelated properties of these connectivity terms, selecting and combining of the molecular connectivity indices in a regression analysis has been difficult for a set of structural congeners [11]. However, the orthogonal connectivity indices that are derived from a procedure called Dominant Component Analysis (DCA) has been introduced by Randić to overcome these deficiencies [11].

A QSAR approach is reported in this paper for 22 chlorinated cyclodiene insecticides and analogs by using multiple regression analysis between an *in vitro* biological parameter and orthogonal connectivity indices. The median inhibitory concentration (IC_{50}) of the compounds on inhibition of TBPS-binding in a GABA-receptor preparation of rat brain P2 membranes is used as the biological parameter in the study. Attention in this study has been focused on building models which can with some high probability predict the toxicological effects of other untested compounds in this category.

MATERIALS AND METHODS

Chemicals

Twenty-two chlorinated cyclodienes and structural analogs were used in this study. Structures of the compounds are given in Figure 1. Details of the nomenclature for these compounds were described by Brooks [13]. Most of the chemicals were obtained from Vesicol Corporation and Chem Service, Inc. Some of the structural analogs were synthesized by Dr. Earl Alley at Mississippi State University.

Biological activity

The median inhibitory concentration (IC_{50}) of each compound for competitively inhibiting the binding of the ligand TBPS at the TBPS-site in a rat brain synaptic membrane preparation was used as the biological parameter. Measurement of this parameter is an *in vitro* assay. This assay has been a frequently used probe to characterize the action of chemicals acting at the GABA receptor. Measurement of this parameter was essentially conducted according to a procedure described by Casida and Lawrence [2, 3] with slight modification.

Molecular connectivity [8, 14]

Molecular connectivity is a method of molecular structure quantitation in which weighted counts of substructure fragments are incorporated into numerical indices. Structural features such as size, degree of branching, unsaturation, heteroatom content and cyclicity are the

elements encoded. The valence molecular connectivity indices are the main descriptors employed in this study. The calculation of the valence molecular connectivity index starts with the reduction of the molecule to the hydrogen-suppressed skeleton or graph. Each atom is assigned an atom descriptor, δ^v, based on the count of valence electrons present, other than those bonded to hydrogen atoms:

$$\delta^v = (Z^v - h)/(Z - Z^v - 1)$$

where Z is the count of all electrons, i.e., the atomic number, and Z^v is the count of the valence electrons, and h is the count of hydrogen atoms bonded to the atom. The valence molecular connectivity indices or *chi* indices are symbolized by ${}^m\chi_t^v$. Substructures for a molecular skeleton are defined by the decomposition of the skeleton into fragments of *a*) atoms (zero order, $m = 0$); *b*) one-bond paths (first order, $m = 1$); *c*) two-bond fragments (second order, $m = 2$); *d*) three contiguous bond fragments (third order Path, $m = 3$, $t = $ P) and so forth. Other fragments include the cluster (three atoms attached to a central atom, $m = 3$, $t = $ C); the path/cluster (equivalent to the isopentane skeleton, $m = 4$, $t = $ PC); the chain fragment (cycles of 3, 4, 5 ... atoms, $m = 3, 4, 5...$, $t = $ CH). For each order and fragment type, a connectivity index is calculated as

$${}^m\chi_t^v = \sum_{i=1}^{Ns} {}^mc_i \quad \text{and} \quad {}^mc_i = \prod_{k=1}^{m+1} (\delta_k^v)^{-1/2}$$

where ${}^mc_i$ is the reciprocal square root of the multiplication of the δ^v values for each atom in a fragment and is called the connectivity subgraph term, and ${}^m\chi_t^v$ is the sum over all the subgraphs, *Ns*, of order m and type t in the entire molecule. Calculation of molecular

connectivity indices was carried out by Molconn-X software obtained from Kier and Hall [15]. This program allows a family of structurally related descriptors to be calculated. The valence molecular connectivity indices used in this study are listed in Table 1. All of the values in Table 1 are different levels of valence molecular connectivity indices, $^{1}\chi^{v}$, $^{2}\chi^{v}$, $^{3}\chi^{v}$, and $^{4}\chi^{v}$, of path-type substructures.

Data analysis

Establishment of correlation models between the molecular connectivity descriptors and the biological activity was accomplished through multiple regression analysis, using the Statistical Analysis System (SAS 6.07) at a workstation of Project Vincent/ULTRIX v4.3A main frame at the Iowa State University Computation Center. PROC RSQUARE and PROC REG subroutines [16] were performed to find optimum the best regression equations.

RESULTS AND DISCUSSION

For the 22 chlorinated cycodienes and their structural analogs, regression models were built on those including no more than three-variable combinations of the prediction descriptors to avoid chance correlation. In a multiple regression analysis, the $\log(1/IC_{50})$ values were screened against all possible two- and three-variable combinations of the simple level (χ) and valence level (χ^{v}) indices. Other variables included the inverses of χ or χ^{v}, squares of χ or χ^{v}, difference variables of ($^{0}\chi$ - $^{0}\chi^{v}$) and ($^{1}\chi$ - $^{1}\chi^{v}$), the third-order cluster index ($^{3}\chi^{v}_{c}$) and the fourth path/cluster index ($^{4}\chi^{v}_{p/c}$). It was found that the three-variable

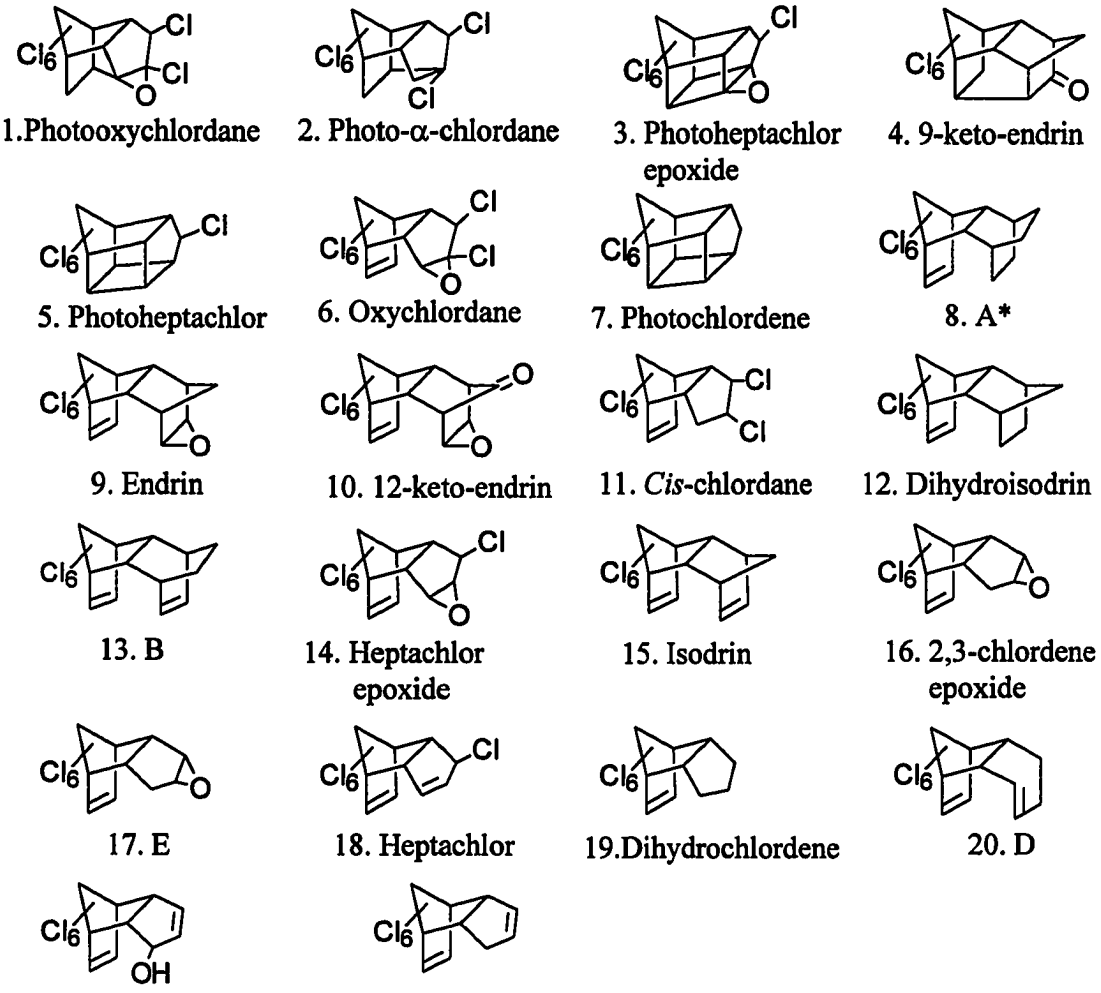

Figure 1. Structures of 22 chlorinated cyclodiene insecticide analogs. Nomenclature refers to Ref. [12]. *A, 5,6,7,8,9,9,-hexachloro-1,2,3,4,4a,5,8,8a-octahydro-1,4-ethano-5,8-methanonaphthalene; B, 5,6,7,8,9,9,-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-ethano-5,8-methanonaphthalene; D, 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-1,4-methanonaphthalene; E, 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,8a-hexahydro-1,4-methanonaphthalene.

Table 1. Values of the valence molecular connectivity indices, $^{1}\chi^{v}$, $^{2}\chi^{v}$, $^{3}\chi^{v}$ and $^{4}\chi^{v}$, for the 22 chlorinated cyclodiene compounds. Numbering of each compound is the same as that in Figure 1.

No.	$^{1}\chi^{v}$	$^{2}\chi^{v}$	$^{3}\chi^{v}$	$^{4}\chi^{v}$
1	8.7680	10.6297	12.0285	10.3763
2	8.5828	10.4554	12.1016	10.2042
3	8.2859	10.4173	12.3273	11.5092
4	8.6141	10.2333	11.5087	10.2111
5	8.1277	10.1851	11.9623	11.0343
6	8.4678	9.6520	10.1281	7.6582
7	7.6229	9.6338	11.4941	10.2760
8	8.8620	9.6280	10.1757	8.3341
9	8.5169	9.5818	10.0797	8.3437
10	8.4819	9.4724	9.9076	8.0236
11	8.3548	9.2945	9.7920	7.4605
12	8.3620	9.2863	9.8730	8.1760
13	8.5455	9.2431	9.7049	7.7861
14	8.0218	8.9668	9.2995	7.3946
15	8.0455	8.9014	9.4159	7.6143
16	7.5169	8.4087	8.7959	6.7961
17	7.8789	8.3990	8.8824	6.8826
18	7.5504	8.2864	8.6058	6.5965
19	7.3789	8.0454	8.6324	6.7118
20	7.5455	8.0393	8.4578	6.3528
21	7.1539	7.8286	8.2270	6.1336
22	7.0455	7.6977	8.2034	6.1165

combinations from $^{1}\chi^{v}$, $^{2}\chi^{v}$, $^{3}\chi^{v}$ and $^{4}\chi^{v}$, which are given in Table 1, showed good correlations with the log $1/IC_{50}$ values. The following equation was the best model found.

$$\text{Log } 1/IC_{50} = 2.47(\pm 0.697)^{2}\chi^{v} - 2.70(\pm 1.07)^{3}\chi^{v} + 1.32(\pm 0.619)^{4}\chi^{v} - 8.53(\pm 2.41) \quad (1)$$

$$n = 22 \quad r = 0.941 \quad s = 0.327 \quad F_{3,18} = 46.1 \quad Q^{2} = 0.825 \quad s_{PRESS} = 0.403$$

where n is the number of compounds, r is the correlation coefficient, s is the standard error of the estimate, F, the Fisher significance value, i.e., the ratio between regression and residual variances. Due to the relatively small set of compounds used, the leave-one-out procedure was employed to validate the model. S_{PRESS} is the standard deviation of predictions and PRESS is the predictive residual sum of squares. Q^{2} is the squared correlation coefficient of predictions [17]. Figures in parentheses are confidence intervals of the regression coefficients at the 95% significance level.

On the other hand, there are intercorrelations or multicollinearity, among $^{1}\chi^{v}$, $^{2}\chi^{v}$, $^{3}\chi^{v}$, and $^{4}\chi^{v}$ as shown by the correlation matrix in Table 2. For example, the correlation coefficient between $^{2}\chi^{v}$ and $^{3}\chi^{v}$ is as high as 0.958. The correlation coefficients for the pairs of $^{2}\chi^{v}$ and $^{4}\chi^{v}$, and $^{3}\chi^{v}$ and $^{4}\chi^{v}$, are 0.917 and 0.985, respectively, which are also very appreciable. The magnitude of an intercorrelation coefficient between a pair of descriptors expresses the degree of overlap of information that they encode for the compounds, and the high intercorrelation may be due to the inherent properties of the molecular connectivity indices. The existence of multicollinearity in a multiple regression model makes it difficult, if not impossible, to disentangle the unique effects of each individual predictor on the response variable [18-20]. The interpretation of the regression coefficient as measuring

Table 2. Intercorrelation matrix between different levels of the valence molecular connectivity index and the biological parameter. $A = \log (1/IC_{50})$.

	A	χ_1^v	χ_2^v	χ_3^v	χ_4^v
A	1				
χ_1^v	0.8188	1			
χ_2^v	0.8327	0.8335	1		
χ_3^v	0.7263	0.6574	0.9584	1	
χ_4^v	0.7219	0.5816	0.9169	0.9849	1

the change in the expected value of the response variable when the corresponding explanatory variable is increased by one unit while all other explanatory variables are held constant is not fully applicable when multicollinearity exists [19]. Moreover, multicollinearity generally leads to instabilities of estimated coefficients that are subject to dramatic changes due to adding or deleting of variables or small changes in data points. However, a procedure to orthogonalize molecular connectivity indices has been introduced by Randić [12, 21] to correct these deficiencies and resolve ambiguities in structure-activity correlations. This procedure has been called the Dominant Component Analysis (DCA) that is analogous to the Gram-Schmit procedure for the orthogalization of vectors [22].

The descriptors ${}^{1}\chi^{v}$, ${}^{2}\chi^{v}$, ${}^{3}\chi^{v}$, and ${}^{4}\chi^{v}$ were converted to orthogonal descriptors by the procedure outlined here. The DCA procedure places emphasis on residuals of intercorrelation between descriptors, that is, the fraction which may represent a unique structural characterization innate to a new particular descriptor, not the parts (small or large) in which the descriptors duplicate one another, to eliminate redundant or overlapping aspects of the descriptor set to be considered in the regression analysis. Even when the intercorrelation coefficient is as high as 0.95 or higher, the two descriptors may differ significantly since each possesses a fraction that represents unique structural information not contained in the other [23]. Tending to be based on examination of mutual relatedness for all pairs of descriptors considered and excluding a descriptor from a multivariate regression because it is highly correlated with another already included, are inappropriate since this only emphasizes the parts of the descriptors which duplicate each other, rather than focusing

Table 3. Orthogonal molecular connectivity descriptors derived from $^{1}\chi^{v}$, $^{2}\chi^{v}$, $^{3}\chi^{v}$ and $^{4}\chi^{v}$, with $^{1}\chi^{v}$ taken as the first orthogonal descriptor, $^{1}\Omega$.

No.	$^{1}\Omega$	R(2/1)	R(3/1)	R(4/1)	RR(3/2)	RR(4/2)	RRR(4/3)
1	8.768	0.4751	0.9062	0.9512	-0.0542	-0.2958	-0.2063
2	8.5828	0.5586	1.2857	1.1132	0.1564	-0.3531	-0.6114
3	8.2859	0.9339	2.0025	2.9538	0.102	0.4861	0.3146
4	8.6141	0.2929	0.641	1.0637	0.0491	0.2952	0.2141
5	8.1277	0.9219	1.8991	2.7643	0.035	0.3439	0.2861
6	8.4678	-0.0847	-0.4976	-1.2253	-0.3257	-1.0021	-0.4642
7	7.6229	1.0735	2.2659	2.9166	0.0952	0.098	-0.0593
8	8.862	-0.6575	-1.102	-1.2605	0.2284	0.467	0.0897
9	8.5169	-0.2232	-0.6272	-0.6284	-0.1752	-0.0415	0.2479
10	8.4819	-0.2839	-0.7414	-0.8854	-0.1667	-0.1391	0.1362
11	8.3548	-0.2848	-0.6468	-1.2192	-0.0702	-0.4705	-0.3546
12	8.362	-0.3031	-0.5777	-0.5167	0.0359	0.28	0.2207
13	8.5455	-0.6017	-1.0493	-1.2376	0.1682	0.3433	0.0655
14	8.0218	-0.1489	-0.5885	-0.6844	-0.2868	-0.2926	0.1811
15	8.0455	-0.2473	-0.5113	-0.5074	-0.0106	0.1428	0.1603
16	7.5169	-0.00406	-0.257	-0.372	-0.2483	-0.3606	0.0495
17	7.8789	-0.5178	-0.7692	-0.9386	0.2786	0.422	-0.0382
18	7.5504	-0.173	-0.5025	-0.6321	-0.1521	-0.177	0.0742
19	7.3789	-0.1752	-0.1922	-0.2074	0.1627	0.2534	-0.0153
20	7.5455	-0.4133	-0.6424	-0.8669	0.1941	0.2192	-0.1014
21	7.1539	-0.0788	-0.2254	-0.3797	-0.0655	-0.172	-0.0638
22	7.0455	-0.0587	-0.0697	-0.2013	0.0496	-0.0464	-0.1283

on those parts of the descriptors which differ [12, 23, 24]. Methodologically, the DCA procedure takes the residuals of intercorrelation between descriptors to be novel descriptors, which are devoid of mutual relatedness.

$^{1}\chi^{v}$, $^{2}\chi^{v}$, $^{3}\chi^{v}$, and $^{4}\chi^{v}$ are made orthogonal as follows: $^{1}\chi^{v}$ is taken as the first orthogonal descriptor, $^{1}\Omega$. The residuals of the correlation pairs, [$^{2}\chi^{v}$, $^{1}\chi^{v}$], [$^{3}\chi^{v}$, $^{1}\chi^{v}$] and [$^{4}\chi^{v}$, $^{1}\chi^{v}$], which are represented by R(2/1), R(3/1) and R(4/1) respectively, are given in the first three columns in Table 3. R(2/1) is selected as the second orthogonal descriptor, $^{2}\Omega$. The process continues to regress R(3/1) and R(4/1) against R(2/1) to obtain the double residuals: RR(3/2) and RR(4/2). The double residual RR(3/2) is designated as the third orthogonal descriptor, $^{3}\Omega$. The next orthogonal descriptor, $^{4}\Omega$, is the triple residual of the regression of RR(4/2) against RR(3/2). Performing a multiple regression analysis involving two three-variable combinations of $^{1}\Omega$, $^{2}\Omega$, $^{3}\Omega$ and $^{4}\Omega$ with log ($1/IC_{50}$) values, the fittest model is obtained as shown by Equation 2 below, which is the three-variable combination of $^{1}\Omega$, $^{2}\Omega$ and $^{4}\Omega$.

$$\text{Log } 1/IC_{50} = 1.35(\pm 0.292)^{1}\Omega + 0.487(\pm 0.316)^{2}\Omega + 1.32(\pm 0.651)^{4}\Omega - 12.9(\pm 2.37) \quad (2)$$

$$n = 22 \quad r = 0.934 \quad s = 0.344 \quad F_{3,18(0.0001)} = 40.1 \quad Q^{2} = 0.815 \quad s_{PRESS} = 0.414$$

In a manner similar to the orthogonalization procedure described above, the prediction descriptors in Equation 1 are also made non-mutually related: $^{2}\chi^{v}$ itself is taken as the first orthogonal descriptor E_1. The next new descriptor, E_2, is derived from the regression of $^{2}\chi^{v}$ against $^{1}\chi^{v}$. Residuals of the regression of $^{2}\chi^{v}$ against $^{1}\chi^{v}$, which represent the part of $^{2}\chi^{v}$ which $^{1}\chi^{v}$ cannot account for, defines E_2. The third new descriptor, E_3, is the double residual

Table 4. Orthogonal molecular connectivity descriptors derived from $^{2}\chi^{v}$, $^{3}\chi^{v}$ and $^{4}\chi^{v}$, with $^{2}\chi^{v}$ taken as the first orthogonal descriptor, E_1, and the comparison of the calculated log(1/IC_{50}) values from Equations 2 and 3 with the observed log(1/IC_{50}) values.

	Orthogonal descriptor			log(1/nanomolar IC_{50})				
No.	E_1	E_2	E_3	obs.	eq.3	residual	eq.2	residual
1	10.6297	-0.0247	-0.2085	-1.46	-1.01	-0.46	-1.04	-0.43
2	10.4554	0.3	-0.6053	-1.67	-1.86	0.19	-1.78	0.11
3	10.4173	0.5807	0.3138	-0.72	-0.84	0.12	-0.77	0.05
4	10.2333	0.0277	0.217	-1.07	-0.80	-0.26	-0.78	-0.29
5	10.1851	0.5509	0.2822	-0.70	-1.06	0.36	-1.04	0.33
6	9.652	-0.5138	-0.4767	-1.54	-1.89	0.35	-2.06	0.52
7	9.6338	0.8785	-0.0636	-2.30	-2.16	-0.14	-2.10	-0.20
8	9.628	-0.4316	0.1081	-1.12	-1.18	0.06	-1.07	-0.05
9	9.5818	-0.4609	0.2435	-1.23	-1.02	-0.21	-1.12	-0.11
10	9.4724	-0.4751	0.1324	-0.64	-1.25	0.62	-1.34	0.71
11	9.2945	-0.3339	-0.3545	-2.46	-2.12	-0.34	-2.16	-0.30
12	9.2863	-0.241	0.2258	-1.70	-1.42	-0.28	-1.40	-0.30
13	9.2431	-0.3468	0.0793	-1.77	-1.59	-0.18	-1.50	-0.26
14	8.9668	-0.3533	0.1687	-1.82	-1.69	-0.13	-1.84	0.02
15	8.9014	-0.1425	0.1612	-1.47	-1.88	0.41	-1.88	0.42
16	8.4087	-0.0514	0.0356	-2.48	-2.50	0.02	-2.63	0.15
17	8.399	0.0492	-0.0229	-2.77	-2.65	-0.12	-2.50	-0.27
18	8.2864	-0.0649	0.0659	-2.54	-2.56	0.01	-2.63	0.09
19	8.0454	0.3096	-0.00974	-3.25	-3.07	-0.17	-2.98	-0.26
20	8.0393	0.1438	-0.0921	-2.63	-3.09	0.46	-2.99	0.36
21	7.8286	0.2171	-0.0703	-3.73	-3.28	-0.45	-3.31	-0.42
22	7.6977	0.3824	-0.1299	-3.40	-3.56	0.15	-3.53	0.12

of the regressions [$^3\chi^v$, $^2\chi^v$] against [$^4\chi^v$, $^2\chi^v$,]. The new model from the combination of the orthogonal descriptors E_1, E_2 and E_3 is:

$$\text{Log } 1/IC_{50} = 0.825(\pm 0.166)E_1 - 0.579(\pm 0.387)E_2 + 1.32(\pm 0.619)E_3 - 9.51(\pm 1.54) \quad (3)$$

$n = 22 \quad r = 0.941 \quad s = 0.327 \quad F_{3,18} = 46.1 \quad Q^2 = 0.825 \quad s_{PRESS} = 0.403$

Equations 2 and 3 explain 87-88% of the variation of the data, giving satisfactory correlations between the log($1/IC_{50}$) values and the orthogonal molecular connectivity descriptors. It was found that all confidence intervals for regression coefficients were higher than 95%. Confidence intervals at 95% significance level are the values in parentheses. Calculated log($1/IC_{50}$) values from both equations and their residuals are given in Table 4, and a comparison of the observed and calculated log ($1/IC_{50}$) values is shown in Figure 2.

In Equation 2, $^1\Omega$, $^2\Omega$ and $^4\Omega$ are making significant contributions to the regression. $^1\Omega$, like $^1\chi^v$, is a summation over the bond terms, $^1\chi^v = \sum(\delta_i^v * \delta_j^v)^{-0.5}$. The valence delta values, δ^v, reflect the atom core charge, the number of *pi* and lone pair orbitals in addition to the non-hydrogen sigma bond, and each of these structural features contributes to attributes such as atomic and molecular volume [8]. Therefore the correlation can be interpreted that log ($1/IC_{50}$) is partially dependent on properties related to $^1\chi^v$. The coefficients of $^2\Omega$ and $^4\Omega$ represent additional structural dependence of log ($1/IC_{50}$) that goes beyond information encoded in $^1\chi^v$, $^2\chi^v$, $^3\chi^v$ and $^4\chi^v$.

Equation 3 has the same structural content as that in Equation 1, but it is orthogonally re-partitioned with the non-mutually related descriptors, E_1, E_2 and E_3. E_2 has a negative

effect on the magnitude of log ($1/IC_{50}$). The $^2\chi^v$ index gives weight to structural features of two-bond lengths with consideration of the importance of heteroatoms in the molecule. The $^2\chi^v$ value is calculated from the sum of all $(\delta_i^v * \delta_j^v * \delta_k^v)^{-0.5}$ terms in a molecule. The magnitude of $^2\chi^v$ is, therefore, dependent not only on the values of δ_i^v, δ_j^v, and δ_k^v in each fragment, but also on the number of the fragments into which the molecule can be dissected. $^3\chi^v$ and $^4\chi^v$ are similar to $^2\chi^v$ but calculated from the sums of $(\delta_i^v * \delta_j^v * \delta_k^v * \delta_l^v)^{-0.5}$ and $(\delta_i^v * \delta_j^v * \delta_k^v * \delta_l^v * \delta_m^v)^{-0.5}$, which are numerical values of three-bond- and four-bond-length fragments, respectively. $^2\chi^v$, $^3\chi^v$ and $^4\chi^v$ may represent properties with related fragment additivity which is close to bond additivity. With respect to the orthogonalized descriptors, E_1 or $^2\chi^v$ represents the property that is attributed to molecular fragment additivity which is close to bond additivity. Clearly, E_2 and E_3 encode structural information that the nonorthogonal descriptors cannot account for. This information is not the same as those additive characteristics of the compounds, but they may be related to other topological or three-dimensional aspects of the molecules.

It is shown that regression coefficients have a constancy, i.e., they are not influenced by inclusion/exclusion of additional variables in comparing the regression equations in Table 5, based on $^2\chi^v$, $^3\chi^v$ and $^4\chi^v$, and based on the orthogonal descriptors, E_1, E_2 and E_3. The stability of the regression coefficient results in the interpretation of the effect of each variable

Table 5. Regression equations for the inhibition concentrations expressed in log ($1/IC_{50}$) of the chlorinated cyclodienes from the valence molecular connectivity indices and orthogonalized connectivity indices. R is the correlation coefficient and *s* is the standard error.

descriptors	χ_2^v	χ_3^v	χ_4^v	Intercept	R	*s*
χ_2^v	0.8248			-9.5142	0.8327	0.4753
χ_2^v, χ_3^v	1.6605	-0.579		-11.4192	0.8698	0.4625
$\chi_2^v, \chi_3^v, \chi_4^v$	2.4727	-2.6988	1.3202	-8.5277	0.9406	0.3268
	E_1	E_2	E_3			
E_1	0.8248			-9.5142	0.8327	0.4753
E_1, E_2	0.8248	-0.5789		-9.5142	0.8698	0.4625
E_1, E_2, E_3	0.8248	-0.5789	1.3202	-9.5142	0.9406	0.3268

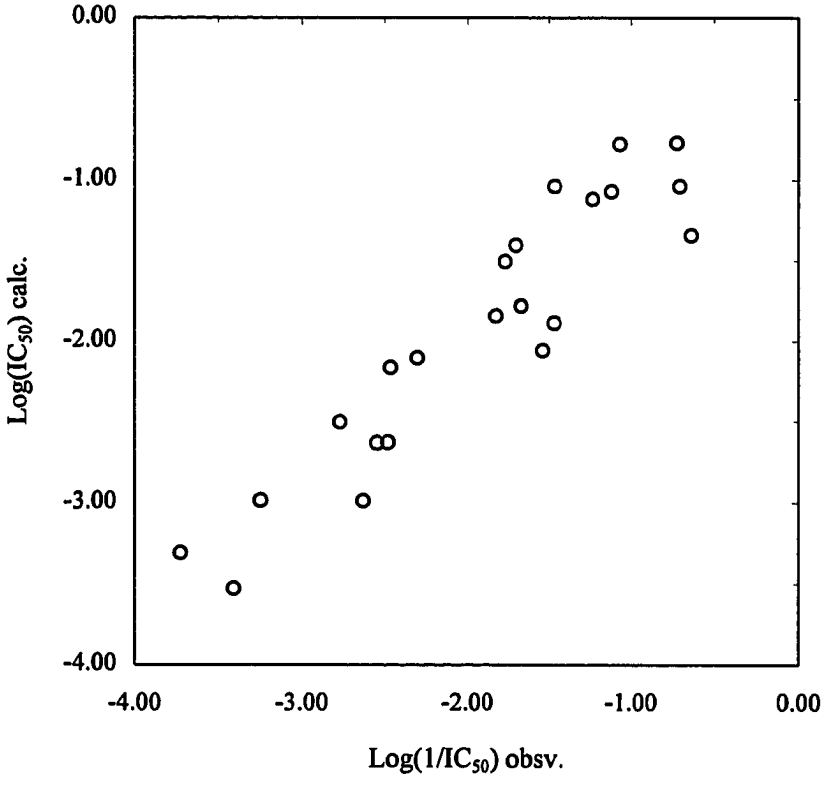

Figure 2. Observed and calculated (eq. 2) $\log(1/IC_{50})$ values.

without ambiguity. But orthogonal descriptors derived from molecular connectivity indices are still classified as structure-cryptic so far because they do not completely reveal the underlying structural factors that govern the property examined [25].

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SUMMARY AND CONCLUSION

The experiments implemented in this dissertation research addressed the structural dependence of biological action of the cyclodiene insecticides and established certain models for prediction of toxicity by physicochemical parameters such as the lipophilic descriptor, k_w, and different levels of valence molecular connectivity indices, which are the integral physicochemical parameters considering a active molecule as a whole.

The data from the *in vitro* TBPS-binding assay showed that many compounds from the cyclodiene family are antagonists, *i.e.,* blockers of the chloride channel of the GABA receptor ionophore complex. The most active among the tested compounds were 12-ketoendrin, photoheptachlor epoxide and Δ-ketoendrin. Dieldrin and endrin, a pair of stereoisomers, showed a thirteen-fold difference in their activities, while aldrin and isodrin, another pair of stereoisomers, showed an eleven-fold difference in activity. Moreover, the epoxide form is more potent than its parent cyclodiene forms, e.g., dieldrin vs. aldrin, heptachlor epoxide vs. heptachlor, chlordene epoxide vs. chlordene. Aldrin *cis*- and *tran*-diols, chlordecone, and mirex, showed extremely low activities, and they were probably not antagonist of the GABA receptor ionophore complex.

Lipophilicity plays an important role in the action of cyclodiene compounds competing with the binding of [^{35}S]TBPS to the GABA receptor in the rat brain P2 membrane preparation. The epoxide or ketone structural congeners, and the non-epoxide non-ketone cyclodiene analogs may bind to different regions, respectively, on the common GABA

receptor, as shown by the relationships between the lipophilicity and the inhibition on TBPS-binding. The epoxide or ketone congeners may bind at a more hydrophilic region, and a negatively correlated linear relationship exists between the inhibition of TBPS-binding and lipophilicity. However, the non-epoxide and non-ketone analogs may bind at a very lipophilic region, and there is a positively correlated linear relationship between them. The opposite relationships between lipophilicity and activity on inhibition of TBPS-binding may indicate specific ligand-receptor interactions, since the system for measuring activity was an *in vitro* assay in this study, and the complicating influences of penetration, metabolism, and excretion were eliminated. The epoxide feature of the cyclodienes seems to be an essential structure requirement for eliciting their high inhibitory activity on the GABA receptor.

Further, the shape or rigidity of cyclodiene compounds appear to be another structural motif needed for their biological action. Quantitative structure-activity relationship (QSAR) analysis by building models showed that topological descriptors, $^{1}\chi^{v}$, $^{2}\chi^{v}$, $^{3}\chi^{v}$, and $^{4}\chi^{v}$, the first-, second-, third- and fourth-valence molecular connectivity indices, respectively, are useful and reliable parameters to predict the biological action for cyclodiene insecticide analogs. The structural dependence of biological activity, *i.e.*, the inhibitory activity on [^{35}S]TBPS binding of the cyclodienes, can be described by a combination among $^{1}\chi^{v}$, $^{2}\chi^{v}$, $^{3}\chi^{v}$, and $^{4}\chi^{v}$ in a multivariate regression model. High correlation coefficients ($r = 0.934$ to 0.941) between the biological response parameter and the explanatory molecular connectivity indices demonstrated that the topological and steric attributes of the cyclodienes are structural characteristics important to their biological activity. Molecular connectivity indices are

simple structural descriptors, which can be easily calculated, but are very reliable predictors even if the clear physical definition of high level indices are not well assigned. In a multiple regression analysis, multicollinearity of molecular connectivity indices can be eliminated by introducing orthogonalized indices, which allow the model to not be influenced by inclusion/exclusion of additional variables and the stability of the regression coefficient, resulting in the interpretation of the effect of each variable without ambiguity.

The chemical shift of the angular proton in the proton-NMR spectra was not proven to be a useful descriptor of the electronic effect for the cyclodienes. This failure may be caused by the lack of sufficiently common parent structure for the compounds tested or because the interference of other factors, such as coupling effects, could not be minimized. Electronic effects probably also contribute to the toxicity of the cyclodienes, but the parameter tested here did not reveal any relationship to the TBPS binding.

This dissertation research has largely focused on the quantitative structure-activity relationship analysis without involvement of chemical synthesis of new compounds based on the QSAR analysis, which could possibly block the chloride channel on the GABA receptor ionophore complex. The information drawn from such studies will benefit our understanding of the structural determinants for the biological action of the classic cyclodiene insecticides, and future approaches could aim at the synthesis of modified cyclodienes-type insecticides, perhaps bearing fewer chlorines in the molecule. A better understanding of cyclodiene QSARs will also contribute to an improved capability to assess the toxicological significance of the ubiquitous environmental residues of the cyclodienes and their degradation products.

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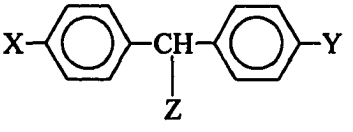
APPENDIX: DIRECTED SYNTHESIS OF NEW BIODEGRADABLE DDT-TYPE INSECTICIDES

ABSTRACT

A new route was designed for chemical synthesis of 1,1-*bis*-(*p*-substituted-phenyl)-2,2-dimethyl-3-halopropanes, a category of new DDT-type insecticide analogs. This approach showed that the key intermediate, 3-halo-2,2-dimethylpropionaldehyde can be synthesized from its methanesulfonate with inorganic halides under a phase-transfer catalyst or anhydrous conditions. Bromo-, chloro- and fluoro-substituted dimethylpropionaldehydes were produced with reasonable yield in their cyclic acetal forms. Preliminary bioassays found that among the six 1,1-*bis*-(*p*-substituted-phenyl)-2,2-dimethyl-3-halopropanes synthesized, β-monofluorinated analogs showed best insecticidal activity against a susceptible strain of house flies, *Musca domestica,* and provided leads for further chemical synthesis approaches.

INTRODUCTION

Many structural analogs of 1,1,1-tricholoro-2,2-*bis*-(*p*-chlorophenyl)-ethane (DDT) have been synthesized for enhanced activity and biodegradability since the initial disclosure of its insecticidal effect. Important commercialized analogs included methoxychlor, perthane, and rhothane. Nitroalkanes such as prolan [1,1-*bis*-(*p*-dichlorophenyl)-2-nitropropane], bulan and dilan were also successful commercialized compounds. Further modification of DDT analogs involved synthesis of diphenylchloronitroethanes and 1,1-*bis*-(4-chlorophenyl)-2,2-dichlorocyclopropanes.



Steric and electronegative characteristics of substituent Z, with an appropriate size combination of X and Y are most important determinants for the insecticidal activity of DDT-type compounds as reviewed by Coats (1). Increasing electronegativity of aliphatic moiety Z may enhance insecticidal activity as shown by neurotoxicological studies (2). Chloro-, dichloro-, trichloro- and nitro-substituted (Z moiety) alkanes have excellent insecticidal activity, while non-substituted alkanes shows only fair insecticidal effect (1, 3). Polyhalogenated alkanes such as trifluoro, pentachloro and chlorodifluoro substituted analogs result in overly modified electronegativity, and they have little insecticidal activity (4). It is evident that proper modification of Z moiety is the key step for approaching new biodegradable insecticides in the DDT-type family.

In this study, synthesis of 3-monohalogenated-2,2-dimethyl-propanes was investigated as biodegradable DDT-type insecticides. A route for synthesis of 1,1-*bis*-(*p*-substituted-phenyl)-2,2-dimethyl-3-halogpropanes is presented. Effort has been focused on the monofluorination of alkanes. The preliminary toxicity test against the susceptible strain of house flies, *Musca domestica*, is also reported in this paper.

EXPERIMENTAL

Chemical synthesis. Synthetic procedures for chemicals are described below. 1,1-*bis*-(*p*-substituted-phenyl)-2,2-dimethyl-3-halopropanes were separated by using silica gel (with 254-nm fluorescent indicator) thin-layer chromatography and further monitored by spraying the diphenyl-zinc chloride reagent (5). Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained with a Nicolet-300 spectrometer in deuterochloroform (CD_3Cl) using tetramethylsilane (TMS) as a internal standard.

3-Hydroxy-2,2-dimethylpropanal (1). To a stirred mixture of isobutyraldehyde (72 g, 1 mol) and 37% formalin (106 g, 1.3 mol), which was cooled with an ice bath, K_2CO_3 (57 g, 0.41 mol) was added, keeping the mixture stirred at room temperature. Stirring was kept at room temperature for an additional 30 minutes after the addition K_2CO_3. The mixture separated into two phases on standing. The organic layer was collected and the aqueous phase was extracted with benzene. The combined collection was dried over Na_2SO_4 (anhydrous), and the solvent evaporated in a rotatory evaporator to give crude 3-hydroxy-2,2-dimethylpropanal (87%). The concentrate was used for making the cyclic acetal in the next step.

2-(1,1-Dimethyl-2-hydroxyethyl)-1, 3-dioxolane (2). 3-Hydroxy-2,2-dimethylpropanal (89 g, 0.87 mol) was dissolved in benzene containing ethylene glycol (52.7 g, 0.85 mol) and *p*-TsOH.H_2O (2.1 g, 0.1 mol) at room temperature. Then the mixture was heated to reflux with a Dean-Stark apparatus until no water was separated. After cooling, the mixture was successively washed with 2N NaOH and brine, dried over Na_2SO_4 and the solvent was

evaporated. The residue was distilled *in vacuo* to give 2-(1,1-dimethyl-2-hydroxyethyl)-1,3-dioxolane (113.9 g, 78%), b.p. 58-60 °C/0.6 mmHg. ^{1}H NMR ($CDCl_3$), showed δ (ppm) 0.963 (s, 6H), 2.63 (s, 1H, going away after adding D_2O), 3.48 (s, 2H), 3.95 (m, 4H), 4.64 (s, 1H).

2-(1,1-Dimethyl-2-methanesulfonyloxyethyl)-1,3-dioxolane (3). Methanesulfonyl chloride (58 g, 0.5 mol) was added dropwise over 45 min. to 2-(1,1-dimethyl-2-hydroxyethyl)-1,3-dioxolane (73 g, 0.5 mol) dissolved in 220 ml of dry pyridine, which was vigorously stirred and cooled with an ice bath. After the addition, the reaction mixture was kept at 0°C for 3 hours. The reaction mixture then was poured into excess water, and the oil which separated was washed repeatedly with fresh aliquots of water before being extracted into methylene chloride. The organic layer was washed with dilute hydrochloric acid (3x 50 ml) and then repeatedly with water until the washings were of neutral pH. The organic layer was dried over anhydrous magnesium sulfate, the solvent evaporated and the residue distilled to give the product as a yellow oil (197 g, 88%), b.p. 113-114°C/0.6 mmHg. 1H NMR ($CDCl_3$), showed δ (ppm) 1.02 (s, 6H), 3.01 (s, 3H), 3.98 (m, 4H), 4.09 (s, 2H), 4.69 (s, 1H).

2-(1,1-Dimethyl-2-fluoroethyl)-1,3-dioxolane (4). In a dry 250-ml three-necked flask, equipped with a sealed stirrer unit, a 100-ml dropping funnel and a short fraction column connected to a downward condenser, was placed a mixture of dry, finely powdered potassium fluoride (59 g, 0.5 mol) and 150 g of dry ethylene glycol. The mantle was heated to 160-170°C and introduced dropwise the 2-(1,1-dimethyl-2-methanesulphonyloxyethyl)-1,3-dioxolane (22.4 g, 0.1 mol). A liquid passed over intermittently at 60-93°C during the course

of the adding. The mantle temperature was allowed to fall to 110-120°C, and a very slow air stream was drawn to the system until there was no liquid passing over at 60-93°C. The combined distillate collected through a fractionating column to give crude 2-(1,1-dimethyl-2-fluoroethyl)-1,3-dioxolane, a yellow liquid (45%), b.p. 92-94°C/39 mmHg. ^{1}H NMR, showed δ (ppm) 0.979 (s, 6H), 3.91 (m, 4H), 4.26 (d, J_{H-F}=47.4 Hz), 4.72 (s, 1H).

2-(1,1-Dimethyl-2-bromoethyl)-1,3-dioxolane (6). In a 50-ml two-necked flask fitted with a stirrer unit and a reflux condenser, were placed methanesulfonate (11.2 g, 0.05 mol), hexadecyltributylphosphonium bromide (1.27 g, 0.0025 mol), toluene (10 ml) and water (3 ml). The reaction mixture was stirred vigorously and heated under reflux for 24 hours. Then, dichloromethane (15 ml) was added, the organic layer separated and the residue extracted with a further portion of dichloromethane. The combined organic extracts were dried over anhydrous magnesium sulfate and the solvent evaporated. The residue was distilled under vacuum to give the product (4.9 g, 69%), b.p. 45-46°C/1.1 mmHg. ^{1}H NMR, showed δ (ppm) 1.04 (s, 6H), 3.40 (s, 2H), 3.91 (m, 4H), 4.74 (s, 1H).

2-(1,1-Dimethyl-2-chloroethyl)-1,3-dioxolane (5). The procedure for synthesis of 2-(1,1-dimethyl-2-chloroethyl)-1,3-dioxolane was very similar to that for 2-(1,1-dimethyl-2-bromoethyl)-1,3-dioxolane, but it required potassium chloride and it had a lower yield (52%). 2-(1,1-Dimethyl-2-chloroethyl)-1,3-dioxolane is a colorless liquid, b.p. 40-40.5°C/1.4 mmHg. 1H NMR, gave δ (ppm) 1.02 (s, 6H), 3.49(s, 2H), 3.91 (m, 4H), 4.74 (s, 1H).

1,1-*bis*-(*p*-Substituted-phenyl)-2,2-dimethyl-3-halopropanes (7-12). The mixture of two molar equivalents of anisole or phenetole with one molar equivalent of a cyclic acetal

was added dropwise to a mixture of 50% concentrated sulfuric acid and 50% glacial acetic acid at 0°C. Once the addition was completed, the reaction mixture was poured over ice. The oil separated was extracted with diethylether. Then the extracts were sequentially washed with 2% $NaHCO_3$ aqueous solution and brine until neutral. The crude product was purified by preparative thin-layer chromatography to give a substituted propane with 60-70% yield. Six substituted propanes were synthesized including:

1,1-*bis*-(*p*-Methoxyphenyl)-2,2-dimethyl-3-fluoropropane (**7**), a brown viscous liquid. ^1H NMR, gave δ (ppm) 1.02 (d, 6H, J=2.1 Hz), 3.76 (s, 6H), 4.01 (d, 2H, J=47.7 Hz), 3.96 (s, 1H), 6.82 (tt, 4H, J=3 Hz), 7.34 (tt, 4H, J=3 Hz).

1,1-*bis*-(*p*-Ethoxyphenyl)-2,2-dimethyl-3-fluoropropane (**8**), a yellow crystal. ^1H NMR, showed δ (ppm) 1.01 (d, 6H, J=1.8 Hz), 1.381 (t, 6H, J=6.9 Hz), 3.99 (q, 4H, J=7.2 Hz), 3.94 (s, 1H), 4.01 (d, 2H, J=47.7 Hz), 6.80 (tt, 4H, J=2.7 Hz), 7.31 (tt, 4H, J=2.7 Hz).

1,1-*bis*-(*p*-Methoxyphenyl)-2,2-dimethyl-3-chloropropane (**9**), a viscous liquid. ^1H NMR, showed δ (ppm) 1.10 (s, 6H), 3.32 (s, 2H), 3.78 (s, 6H), 4.04 (s, 1H), 6.83 (tt, 4H, J=3 Hz), 7.38 (tt, 4H, J=3 Hz).

1,1-*bis*-(*p*-ethoxyphenyl)-2,2-dimethyl-3-chloro-propane (**10**), a viscous liquid. ^1H NMR, showed δ (ppm) 1.09 (s, 6H), 1.39 (t, 6H, J=6 Hz), 3.32 (s, 2H), 4.00 (q, 4H, J=6 Hz), 4.02 (s, 1H), 6.82 (tt, 4H, J=2.7 Hz), 7.36 (tt, 4H, J=2.7 Hz).

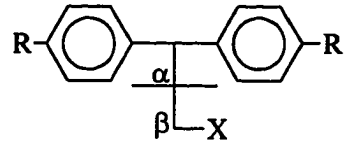
1,1-*bis*-(*p*-Methoxyphenyl)-2,2-dimethyl-3-bromopropane (**11**), a viscous liquid. ^1H NMR, gave δ (ppm) 1.11 (s, 6H), 3.29 (s, 2H), 3.77 (s, 6H), 4.04 (s, 1H), 6.84 (tt, 4H, J=2.4 Hz), 7.39 (tt, 4H, J=2.4Hz).

1,1-*bis*-(*p*-Ethoxyphenyl)-2,2-dimethyl-3-bromopropane (**12**), a viscous liquid. ^{1}H NMR, δ (ppm) 1.12 (s, 6H), 1.38 (t, 6H, J=6.9 Hz), 3.27 (s, 2H), 3.98 (q, 4H, J=6.9 Hz), 4.0 (s, 1H), 6.80 (tt, 4H, J=2.4 Hz), 7.35 (tt, 4H, J=2.4 Hz).

Bioassay. Insecticidal activity of 1,1-*bis*-(*p*-substituted-phenyl)-2,2-dimethyl-3-halopropanes was measured against the susceptible strain of house flies, *Musca domestica*, at room temperature (26 °C), by topical application. Known concentrations of the compounds were applied with a syringe applicator to the thorax of CO_2 anesthetized house flies. Ten insects received each treatment, and the treatments were conducted in triplicate. Methoxychlor was used as a positive control for toxicity comparison. Mortality was recorded at 24 hours following exposure. LD_{50} values were calculated using the Spearman-Karber procedure (6).

RESULTS AND DISCUSSION

Insecticidal propanes, i.e., 1,1-*bis*-(*p*-substituted-phenyl)-2,2-dimethyl-3-halopropanes (**I**), can be synthesized from the condensation of two molar equivalents of *p*-substituted benzene with one molar equivalent of 3-halo-2,2-dimethylpropanal.



I (X=F, Cl, Br)

The key intermediate is 3-halo-2,2-dimethylpropanal, which can be produced by displacing the hydroxyl group in 3-hydroxy-2,2-dimethylpropionaldehyde. The route used for synthesis of the aldehyde is shown in Figure 1. The aldehyde (**1**) was produced by the aldol condensation of isobutylaldehyde and formaldehyde (7, 8). The aldehyde group was then protected by converting it into the cyclic acetal (**2**). Compound **2** could theoretically be directly fluorinated by diethylaminosulfur trifluoride (DAST) (9, 10) to give the desired acetal, but it was not successfully synthesized by using this reagent. When the hydroxyl group in **2** is derivativized to its methansulfonate (**3**), which acts as a good leaving group, it can be readily converted into alkyl halides by reaction with inorganic halides under phase-transfer catalyst (PTC) conditions (11, 12) to give halogenated cyclic acetals, **4**, **5** and **6**. This study showed that reactions under the PTC reagent, hexadecyltributylphosphonium bromide, gave higher yield than crown ethers, another type of PTC reagent (13). However, the methansulfonyloxyl group cannot be displaced by the fluorine anion under PTC

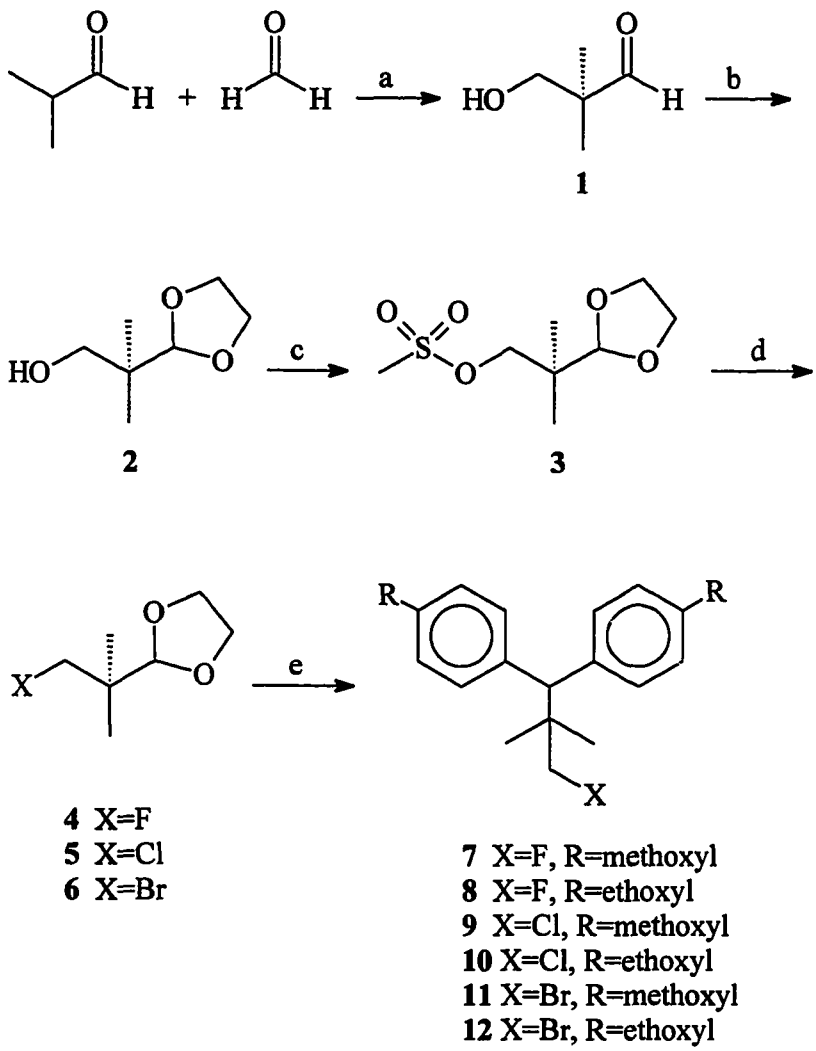
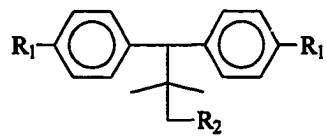


Figure 1. Synthetic scheme. a: K_2CO_3, 0 °C; b: ethylene glycol, *p*-TsOH·H_2O, benzene, reflux; c: methanesulfonyl chloride, pyridine, 0 °C; d: **4**, KF, ethylene glycol, 160-170 °C; **5** and **6**, KCl or KBr , hexadecyltributylphosphonium bromide, toluene and water; e: two molar equivalents of anisole or phenetole, 1:1 H_2SO_4 and AcOH, 0 °C.

Table 1. Toxicity of substituted propanes to house flies by topical application:



Compound	R1	R2	LD_{50} (μg per fly)	95% confidence interval
12	C_2H_5O-	Br	>120	-
11	CH_3O-	Br	>120	-
9	CH_3O-	Cl	>90	-
10	C_2H_5O-	Cl	>75	-
8	C_2H_5O-	F	15	11-20
7	CH_3O-	F	9.4	7.5-12
methoxychlor	-	-	3.6	2.6-5.0

conditions. The fluorination reaction was achieved through refluxing the methanesulphonate with potassium fluoride in anhydrous ethylene glycol. Final step of the sy thetic scheme is the condensation of two mole of anisole or phenetole with one mole of a cyclic acetal to produce substituted propanes, compounds **7** to **12**.

Results of the preliminary toxicity test against the susceptible strain of house flies, *Musca domestica,* for compounds **7** to **12** are shown in Table 1. As expected, the fluorinated propanes, **7** and **8**, have much higher insecticidal activity against house flies than the chlorinated and brominated analogs, **9** to **12**. Very low insecticidal activity of compounds, **9** to **12**, may be caused by the overly bulky effect of the substituent, i.e., the 2,2-dimethyl-3-halogen-moiety in the propanes. Compound **7** showed highest activity among the six propane analogs, and the potency is very close to that of methoxychlor. Neither compound **7** nor **8** showed higher insecticidal activity than methoxychlor. The position of the fluorine atom introduced (α or β position, see structure **I** above) may have significant effect on the insecticidal activity of the compounds, and the α-position fluorinated propanes may have higher activity than these β-fluorinated analogs according to the theory of the action for DDT-type insecticides. On the other hand, toxicity may be different against different insect species.

In conclusion, monofluorinated alkanes for the DDT-type insecticides may contribute to a number of new biodegradable insecticides. Research remains to be done on the chemical synthesis of α-fluorinated alkanes, extended bioassay on different insect species, and toxicological evaluation on the chemicals synthesized.

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